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GENETICS

A Periodical Record of Investigations Bearing on Heredity and Variation

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SHRIVELLED ENDOSPERM IN SPECIES CROSSES IN WHEAT, ITS CYTOLOGICAL CAUSES AND GENETICAL EFFECTS.[1]

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Received May 1, 1929

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INTRODUCTION

In many species crosses it has been found that the second and later generations are composed chiefly of individuals identical with or very similar to the pure species. The hybrids with various combinations of the parental characters, which should constitute nearly the whole F_2 if ordinary genetic principles apply, are missing or scantily represented. In general, the greater the sterility of the F_1, the more the F_2 plants resemble the pure species. In cases in which the species differ in chromosome number this result may be attributed to the death of gametes with intermediate chromosome numbers, or to their inability to function normally owing to the unbalanced condition of the chromosomes; it may, of course, also be due to mortality among zygotes with intermediate numbers.

An additional factor was suggested by the work of THOMPSON and CAMERON (1928) on the chromosome numbers of functioning gametes of species hybrids in wheat. They observed indications that shrivelling of the endosperm of hybrid seeds might play a large part in the non-appearance of expected hybrid types, and that the shrivelling was in turn due

[1] This investigation has been carried on with the aid of grants from the NATIONAL RESEARCH COUNCIL of Canada.

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to chromosome conditions in the endosperm. The present paper gives the results of an investigation to determine the exact relations among chromosome numbers, shrivelling of the endosperm, and elimination of hybrid types, in crosses between 21- and 14-chromosome species of wheat (*vulgare* series × emmer series).

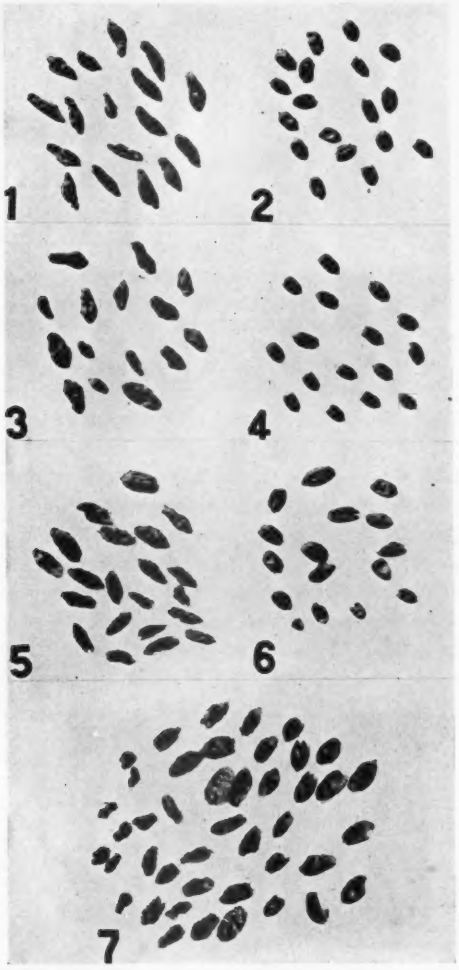
The endosperm is a unique tissue in that it results from the fusion of 2 female cells and 1 male cell. Its chromosome conditions are, therefore, different from those of any other tissue of normal plants. If the chromosome numbers of the parents are different the number present in the endosperm will depend on the direction of the cross. If the parent with the larger number is female, the endosperm will have twice the *larger* number plus the smaller; whereas if it is male, the endosperm will have twice the *smaller* number plus the larger. The totals will therefore be very different in endosperms of reciprocal crosses. Consequently, if the chromosome number has anything to do with endosperm development, we may expect to find a difference between the endosperms of reciprocal crosses.

SAX (1921) has recorded the weight of F_1 grains of several crosses between 14- and 21-chromosome wheats and their reciprocals. He found that the grains were consistently a little heavier when the 14-chromosome species was female than when it was male. He mentioned no difference between reciprocal crosses in regard to the wrinkling of grains, and, in fact, stated that in all cases they were small and wrinkled. Later, the same author (SAX 1922) reported that there is little or no correlation between the weight of F_2 seeds (borne on F_1 plants) and the height or sterility of the F_2 plants which they produced. As between the wrinkled or plump condition and F_2 sterility he reported a correlation coefficient of 0.24 when only those plants which bore heads were considered. If all sterile plants and ungerminated seeds were taken into consideration the correlation would be higher. The observations of WATKINS (1927) and of THOMPSON and CAMERON (1928) differ from those of SAX in that they found the F_1 grains to be plump if *vulgare* was used as the female parent, but invariably wrinkled if it was male. The plump seeds germinated much better than the wrinkled ones.

EXPERIMENTAL WORK

Direct Crosses, F_1

The F_1 seeds of many crosses between *vulgare* and different members of the emmer series have been examined and a consistent difference between reciprocal crosses has been observed. When the *vulgare* (21-chromosome) parent is male all the seeds are wrinkled and unhealthy in appear-



EXPLANATION OF FIGURES

FIGURE 1.—F_1 grains of *dicoccum* ♀ × *vulgare* ♂.
FIGURE 2.—F_1 grains of reciprocal cross.
FIGURE 3.—F_1 grains of *durum* ♀ × *vulgare* ♂.
FIGURE 4.—F_1 grains of reciprocal.
FIGURE 5.—Grains of (*dicoccum* × *vulgare*) F_1 ♀ × *vulgare* ♂.
FIGURE 6.—Grains of reciprocal backcross.
FIGURE 7.—F_2 Grains of *durum* × *vulgare*.

ance in every case, but when it is female they are plump and healthy, though smaller than in the pure species. The difference may be seen by comparing figure 1 (*dicoccum* ♀ ×*vulgare* ♂) with figure 2 (the reciprocal) or figure 3 (*durum* ♀ ×*vulgare* ♂) with figure 4 (the reciprocal). The grains illustrated are those which result directly from the cross pollination and which will grow into F_1 plants. The wrinkled grains of emmer ×*vulgare* are usually slightly larger than those of the reciprocal cross. This does not indicate greater vigor but is apparently due to the fact that grain size is determined chiefly by the female parent, and pure emmer has larger grains than pure *vulgare*. Though the plump seeds of *vulgare* ×emmer are slightly smaller, their greater health is shown by the fact that they germinate much better.

If many F_1 plants are required in order to carry on breeding work on a large scale there is a distinct advantage in using the *vulgare* as the female parent. This advantage more than counterbalances the fact that it is easier to get sufficient pollen from the *vulgare* parent.

The relation of chromosome number to endosperm condition in these crosses is shown in table 1. It is clear from this table that when there are 2 sets of the extra 7 *vulgare* chromosomes, the endosperm is plump (though smaller than in pure *vulgare*); but when there is only 1 set the endosperm is wrinkled. And we know from conditions in the pure species that when there are 3 sets (pure *vulgare*) or none (pure emmer) the endosperm is plump and large.

TABLE 1

Chromosome numbers and condition of endosperm in reciprocal crosses.

	Vulgare ♀	EMMER ♂	TOTAL	CONDITION OF ENDOSPERM
All chromosomes contributed	21+21	14	56	plump
Extra 7 *vulgare* chromosomes	7+7	0	14	
	Vulgare ♂	emmer ♀		
All chromosomes contributed	21	14+14	49	wrinkled
Extra 7 *vulgare* chromosomes	7	0+0	7	

When these crosses are carried on to the next generation the F_2 grains (borne on F_1 plants) present a great mixture of plump, wrinkled, shrivelled, large and small (figure 7). They have a corresponding variability in chromo-

some number. It is not possible, however, to investigate by direct microscopic examination, the exact relationship between chromosome numbers and endosperm conditions in such seeds. Nor can this be done by planting grains of the different kinds and determining the chromosome numbers of the offspring. The number of chromosomes in any plant is the sum of the number in the egg and sperm, but a knowledge of this sum does not enable us to decide how many were in the endosperm. In the case of backcrosses, however, the exact number in the endosperm can be determined indirectly. Consequently, the results obtained with later generations of direct crosses are deferred until those obtained with backcrosses are reported.

Backcrosses

Methods and Materials

In the backcross the sum of the numbers of chromosomes contributed by the two gametes can be determined (by examination of the offspring); the number contributed by one gamete (that of the pure parent) is known; consequently the number contributed by the other can be calculated. Knowing the number in each of the two gametes, it is easy to find the number in the endosperm.

Accordingly, 3 kinds of F_1 were backcrossed reciprocally with the parental types. The kinds of F_1 used were crosses of *vulgare* with *durum* (Iumillo), *dicoccum* (Vernal), and *persicum* (Black Persian). For each kind of F_1 there were four kinds of backcrosses:

(1) F_1 female × *vulgare* male.
(2) F_1 " × emmer "
(3) *vulgare* female × F_1 male.
(4) emmer female × F_1 male.

The condition of the endosperm of each grain obtained was noted (plump, wrinkled, shrivelled, large, small). Several or many of each kind were sown, and the chromosome numbers of the resulting plants were determined. This was done by means of smear preparations of pollen mother cells. It was only necessary to determine the number of unmated (univalent) chromosomes at the reduction divisions. The F_1 gamete contributes $14+x$ chromosomes where x is some number from 0 to 7. If the pure parent is emmer, its gamete contributes 14. The two sets of 14 mate in the pollen mother cells and leave the x unmated. These can be recognized by their form and behaviour. If the pure parent is *vulgare* its gamete contributes $14+7$, and the offspring has $(14+x)+(14+7)$. Again the two sets of 14 mate, the x mate with x from the 7,

leaving 7 − x unmated. Therefore the number of univalents actually seen in this backcross will be 7 − x.

Experimental Results

Endosperm conditions in relation to the direction of crossing. The condition of the endosperm in many grains from reciprocal backcrosses is given in table 2. This classification was made by one who knew nothing of the significance of the work but was simply asked to classify the grains according to size and degree of shrivelling.

TABLE 2

Endosperm condition of seeds resulting from backcrosses.

BACKCROSS \ ENDOSPERM	LARGE PLUMP	SMALL PLUMP	LARGE WRINKLED	SMALL WRINKLED	SHRIVELLED	PERCENT OF SUCCESSFUL POLLINATIONS
vulgare ♀ × F_1(*vulgare* × *dicoccum*) ♂	13	25	7	5	1	14.44
reciprocal	8		46	24	45	32.31
vulgare ♀ × F_1(*vulgare* × *persicum*) ♂	8	13	2	4		9.92
reciprocal			71	20	41	35.52
vulgare ♀ × F_1(*vulgare* × *durum*) ♂	32	48		10	7	
reciprocal	19	7	70	28	41	33.96
dicoccum ♀ × F_1 ♂	65		27			30.92
reciprocal	28	16	49	33	6	36.64
persicum ♀ × F_1 ♂	21	7	11	5		29.50
reciprocal	8	12	18	15	3	35.33

It will be observed that in every case in which *vulgare* was the female parent the great majority of the grains are plump while in the reciprocals nearly all are wrinkled or shrivelled. Figure 5 shows a typical group of seeds of (*vulgare* × *dicoccum*) F_1 ♀ × *vulgare* ♂ and figure 6 of the reciprocal backcross. A few wrinkled seeds do occur when *vulgare* is female and a few plump ones when it is male. The results are strikingly similar for the three different kinds of F_1 (*vulgare* × *dicoccum*, *vulgare* × *persicum* *vulgare* × *durum*). It is perhaps significant that in those cases in which nearly all the seeds are plump (*vulgare* ♀) the percentage of pollinated flowers which set seeds is low (9.92 and 14.44 percent in contrast to about 35 percent in reciprocals). This may be due to abortion and non-functioning of pollen in F_1, though large quantities were used in pollination. It may also be possible that seeds which correspond to the wrinkled ones of other crosses cannot form at all.

The lower part of the table shows that in backcrosses with the emmer parent a much better lot of seeds is obtained when the pure parent is female. This result is in contrast to that obtained in direct crosses, for in them all seeds are wrinkled when the emmer is female.

The importance of the wrinkling and shrivelling in relation to the elimination of hybrid types becomes evident when the different kinds are planted. A large proportion of the wrinkled and shrivelled seeds fail to germinate or die at an early stage, whereas the plump ones germinate well. This is true even though the plump ones be small and the wrinkled ones large. The results are given in table 3. Of course it is possible that the same chromosome condition which makes the endosperm shrivelled may make the associated embryo weak.

TABLE 3

Germination of the different kinds of seeds.

BACKCROSS	ENDOSPERM	LARGE PLUMP	SMALL PLUMP	LARGE WRINKLED	SMALL WRINKLED	SHRIVELLED
F_1 ♀ × *vulgare* ♂	planted			43	18	10
	died			17	15	9
vulgare ♀ × F_1 ♂	planted	20	27	7	12	
	died	2	2	1	1	
F_1 ♀ × emmer ♂	planted	15	3	15	5	
	died	2	0	5	5	
emmer ♀ × F_1 ♂	planted	10	10	15	10	
	died	1	0	2	2	

In connection with this table it may also be noted that in those backcrosses in which the majority of the grains are plump those which are wrinkled germinate surprisingly well. This may indicate that in these cases the wrinkling is not usually due to constitutional causes as in the other cases, but to environmental ones. Such outside influences may cause a proportion of any lot of seeds to be wrinkled.

Endosperm conditions and chromosome numbers. The results obtained in determining the chromosome numbers of the F_1 gametes which functioned to produce many backcross seeds are given in table 4. The numbers shown are those of the extra 7 *vulgare* chromosomes, so that the total number in any gamete is obtained by adding 14 to the number shown. For the backcrosses with emmers the chromosome numbers given are those actually seen as univalents; for the backcrosses with *vulgare* the

number given is 7 minus the number of the univalents seen, since, as explained under "methods," each extra chromosome contributed by the F_1 gamete will mate with one of the extra 7 from the *vulgare* gamete.

TABLE 4

Number of extra 7 vulgare chromosomes in F_1 reproductive cells functioning to produce various types of backcross seeds.

BACKCROSS \ ENDOSPERM	LARGE PLUMP	SMALL PLUMP	LARGE WRINKLED	SMALL WRINKLED	SHRIVELLED
vulgare ♀ × F_1(*dicoccum*) ♂	6,6,7,7,	0,0,0,1,1,2,2	2,3,4,4,	2,3,3	
vulgare ♀ × F_1(*persicum*) ♂	0,6,7,7,	0,0,0,1,1,2,4	1	3,3	
vulgare ♀ × F_1(durum) ♂	5,6,7,7,7,7,7	0,0,0,0,0,0 1,1,1,2,2,3		4,4	
F_1(*dicoccum*) ♀ × *vulgare* ♂	6,7		0,1,1,2,3	1,2,2,2, 3,3	2,2,2,4
F_1(*persicum*) ♀ × *vulgare* ♂			1,1,2	1,3,4	3,4
F_1(durum) ♀ × *vulgare* ♂	2,3,4,4,4 5,5,5,5,7		0,0,0,0,0 1,2,2,2,2,2 3,4		
dicoccum ♀ × F_1 ♂	0,0,0,1,1,2,2 7		5,6,6,7,7,7		
persicum ♀ × F_1 ♂	0,0,5	0,1	5,5,6,6		
durum ♀ × F_1 ♂	0,0,01		3,5,5	0,4	
F_1 ♀ × *dicoccum* ♂	0,0,0,7	2,2,3,6,7,7	2,2,3,3,3,3	3,4,4,5	
F_1 ♀ × *persicum* ♂	0,0,0,0,0	0,1,1,2		2,3,3,5	
F_1 ♀ × durum ♂	0,0,0,0,1,1 2,6	4,6,7		3,4	

A glance at this table is sufficient for one to observe that there is a definite relationship between the condition of the endosperm and the chromosome number of the F_1 gamete (and hence of the endosperm itself). The relationship becomes clearer when the numbers in the gametes are translated into the numbers in the endosperms. To do this it is necessary to remember that the chromosome contribution of the female gamete is doubled in the endosperm while that of the male is single. Thus in the first section of the upper row of the table, since the female *vulgare* gametes

have 7 extra chromosomes, the female contribution is 2×7; the male gametes have 6, 6, 7, 7. Therefore the totals are 20, 20, 21, 21. In other words there are 3 complete or nearly complete sets of 7 extra chromosomes.

TABLE 5

Usual number of extra 7 vulgare chromosomes in different types of endosperm.

ENDOSPERM / BACKCROSS	LARGE PLUMP	SMALL PLUMP	LARGE WRINKLED	SMALL WRINKLED	SHRIVELLED
vulgare ♀ × F_1 ♂	3 sets of 7 complete or nearly so	2 complete sets	2 complete plus several	2 complete plus several	
F_1 ♀ × *vulgare* ♂	3 sets or 1 complete and 2 nearly so		1 set or 1 set and few doubled	1 set and several doubled	1 set and several doubled
emmer ♀ × F_1 ♂	0 or few single		1 set complete or nearly so		
F_1 ♀ × emmer ♂	0 or few	2 sets or few double	several doubled	several doubled	

When, in this way, the numbers throughout the table are turned into the numbers in the endosperms and the numbers for similar backcrosses combined, the total results are as shown in table 5. From this simplified table the following correlations become evident.

(1) The seeds are plump and large when the endosperm contains either (a) 3 complete or nearly complete sets of the 7 *vulgare* chromosomes or (b) none or few of them. Condition (a) is the same as or approaches that in pure *vulgare*, (b) that in pure emmer.

(2) They are plump but likely to be small when the endosperm contains 2 sets, complete or nearly so, or a double dose of one or 2 chromosomes.

(3) They are wrinkled when there is 1 set, complete or nearly so.

(4) The shrivelling is increased by the doubling of incomplete sets.

(5) If the 7 be designated a, b, c, etc., the addition of, say, a and b, does not usually produce much effect, but 2a's and 2b's usually have a pronounced effect. Also 3a's and 3b's have a great effect even when one set is complete.

Of course there are individual cases which do not fit into the scheme but this is to be expected since environmental factors also induce wrinkling,

and there may be differences depending on which of the 7 are present or absent.

Another important point which affects the proportion of plump and shrivelled seeds is the failure of many gametes to function. THOMPSON and CAMERON (1928) and SAX (1928) have shown that most of the F_1 male gametes which produce offspring have 0 or 1 *vulgare* chromosomes; some have 6 or 7; very few have intermediate numbers. The female F_1 gametes are less subject to elimination. Table 4 confirms these findings. It must be noted, however, that the plants studied are not a representative sample; on the contrary disproportionately large numbers of wrinkled and shrivelled seeds were deliberately chosen.

On the basis of proportions of functioning gametes with different chromosome numbers, as reported by these authors and confirmed in this paper, the relative frequencies of the main kinds of chromosome combinations in the endosperm and therefore of the degrees of shrivelling, is shown in table 6 for direct and backcrosses. In this table, x is between 1 and 6.

TABLE 6

CROSS OR BACKCROSS	ENDOSPERM				
	THE 7 *vulgare* CHROMOSOMES			FREQUENCY	CONDITION
	FEMALE	MALE	TOTAL		
vulgare ♀ × emmer ♂	7+7	0	diploid	all	plump
emmer ♀ × *vulgare* ♂	0+0	7	haploid	all	wrinkled
vulgare ♀ × F_1 ♂	7+7	0	diploid	many	plump
	7+7	7	triploid		plump
	7+7	x	diploid+x	few	wrinkled
F_1 ♀ × *vulgare* ♂	7+7	7	triploid	rare	plump
	0+0	7	haploid	some	wrinkled
	x+x	7	haploid+2x	some	wrinkled or shrivelled
emmer ♀ × F_1 ♂	0+0	0	absent	many	plump
	0+0	7	haploid	some	wrinkled
	0+0	x	x	few	wrinkled
F_1 ♀ × emmer ♂	7+7	0	diploid	rare	plump
	0+0	0	absent	some	plump
	x+x	0	2x	many	wrinkled or shrivelled

Direct Crosses, F_2

It has already been pointed out that the exact chromosome number in the endosperm of an F_2 seed cannot be determined by finding the number in the plant which it produces. The latter is the sum of the number in the two gametes, whereas one needs to know the number in each gamete. Nevertheless, some information can be obtained in that way and it was considered desirable to secure whatever information was obtainable, even though it was inexact. Accordingly the chromosome situation was determined in a number of F_2 plants of *vulgare*×*dicoccoides*, which grew from plump and from wrinkled seeds. The results are given in table 7.

In so far as these results can be interpreted, they agree with those already described in this paper, and with the results previously reported regarding the chromosome numbers of functioning germ cells. In those cases in which the plant had 21 chromosomes (bivalents+univalents) and the seed was plump, it was probably the egg which contributed the 7 *vulgare* chromosomes; but where the seed was shrivelled it was probably the sperm which contributed the 7. Where the seed was wrinkled though the extra chromosomes were few the latter were probably contributed by the egg.

TABLE 7

Chromosome numbers of F^2 vulgare×dicoccoides produced by different kinds of seeds.

CHROMOSOMES / SEEDS		PLUMP	LARGE WRINKLED	SMALL WRINKLED
Bivalents	Univalents			
14	0	5		
14	1	4		1
14	2	4	1	1
14	3	2	2	3
14	4		1	3
14	5		1	2
14	6	2		1
14	7	1		
15	6		1	
16	5	2		1

DISCUSSION

From the results reported it is clear that the condition of the endosperm depends on its chromosome content, and that many zygotes must fail to develop because of the chromosome combinations in their endosperms. In general the endosperm is plump when the 7 *vulgare* chromosomes

are absent or diploid or triploid or nearly so. It is likely to be wrinkled or shrivelled when (a) haploid for all or many of the 7, (b) diploid or triploid for some only. The farther the chromosome situation departs from the complete absence or diploidy or triploidy of the *vulgare* chromosomes the more severe is the shrivelling.

The results throw light on the non-appearance of many theoretically expected chromosome and genetic types in F_2 and later generations of ordinary crosses. The types which fail to appear are for the most part those which would be expected to have shrivelled endosperms when they were seeds, and therefore fail to germinate. Furthermore, the injurious effect on the endosperm is likely to be more severe in direct crosses than in backcrosses since in the latter the contribution of one parent must be either a complete set or none at all, whereas in ordinary hybrids both male and female gametes may contribute incomplete sets. And in the missing types both must contribute such incomplete sets.

TABLE 8

Chromosome conditions in the endosperm of seeds from which some typical missing hybrids would have to develop.

HYBRIDS WITH	*vulgare* CHROMOSOMES (a, b, c, AND SO FORTH) IN		
	FEMALE NUCLEI	MALE NUCLEUS	ENDOSPERM
16 bivalents	$2(a+b)$	$a+b$	$3a+3b$
16 bivalents+1 univalent	$2(a+b)$	$a+b+c$	$3a+3b+c$
17 bivalents+2 univalents	$2(a+b+c)$	$a+b+c+d+e$	$3a+3b+3c+d+e$

KIHARA (1924) showed that all the hybrids which develop (other than those with 14 bivalents only) have the chromosome formula $(14+x)$ bivalents$+(7-x)$ univalents. If there are more than 14 bivalents there are enough univalents to make the total 21. A complete set of the 7 *vulgare* ones must be present. A missing type with $14+x$ bivalents would have to result from the fusion of 2 gametes both of which had the same *vulgare* chromosomes (x in number). The endosperm would therefore have three of each kind represented in the x. If there were univalents in addition to the $14+x$ bivalents the endosperm condition would still be very much unbalanced unless there were enough univalents to complete the set of 7. A few examples of the missing types and the chromosome conditions in their endosperms are shown in table 8, a, b, c, etc. representing *vulgare* chromosomes. Such endosperms are likely to be more severely shrivelled (if they can develop at all) than any which can be produced in

backcrosses since in the latter none can have three of one kind unless it has a complete set.

It is possible, of course, that the unbalanced chromosome condition which injuriously affects the endosperm also affects the embryo independently of the endosperm. It may be expected, however, that the triploid endosperm would be more severely affected than the diploid embryo. If there is this independent effect, such embryos are subject to a double menace, (1) the unbalance in their own chromosome complement and (2) the shrivelled endosperm.

In practical breeding of wheat, carried on in order to produce better varieties, special attention should be paid to the shrivelled seeds. The natural tendency is consciously or unconsciously to select the better seeds for planting. But this obviously does not give a random sample of the different genotypes. In any study in which an attempt is made to analyse the genetic situation in order to guide practical efforts, special attention must be given to the poor seeds. From the practical standpoint the shrivelled seeds are just those in which the combinations of characters desired by the breeder are likely to occur. The plump ones are likely to have the complete *vulgare* set of chromosomes or none of them. Moreover, if they are planted under ordinary field conditions most of the shrivelled ones will fail to germinate. Planting in the greenhouse under the best controlled conditions of temperature, soil, moisture, etc. followed by transplanting to the field, would appear to be desirable.

The attention of practical breeders may also be directed to the superiority of the backcross. By its use types can be obtained which would never appear in direct crosses. Without the use of the backcross a real genetic analysis of species crosses in wheat would appear to be out of the question. Genetic conclusions based on the small proportion of chromosome combinations which appear in direct crosses would seem to be quite unreliable. In the backcross a larger proportion of the gametes, particularly the female ones, function.

The conclusions drawn from this work on crosses between 14- and 21-chromosome wheats may be illuminating also with respect to other crosses. The present writer has found it extremely difficult to cross *T. monococcum* (7 chromosomes) with species of the emmer series when the former is female but quite easy when it is male. Numerous attempts to cross *monococcum* female with members of the *vulgare* series yielded no results, but the reciprocal cross was occasionally successful. BLEIER'S recent reviews of the literature (1928) show that other investigators have had similar experiences. When the parent with the larger chromo-

some number is female all its chromosomes are represented twice in the endosperm, whereas if it is male they are single. In crosses between a 7-chromosome species of Aegilops (*A. speltoides*) and various species of wheat exactly similar results have been obtained. Also it is well known that rye which has 7 chromosomes readily serves as male parent but not as female in crosses with certain varieties of *vulgare* wheat. MEISTER and TJUMJAKOFF (1928) are the only investigators who have had any success in using rye as female parent in this cross and they report only 2.5 percent successful pollinations, whereas in the reciprocal they found more than 60 percent successful.

Outside the cereals reports of species crosses which give sufficient information are not numerous enough to enable one to decide whether these conclusions are of general application. Reports have been noted, as in connection with *Nicotiana* (EAST 1928), of cases in which the species with the larger chromosome number must serve as female if success is to follow. In other cases there appear to be exceptions, but it is possible that in these cases the seeds are small and the endosperm does not play as important a part as in cereals.

SUMMARY

1. In crosses between 14- and 21-chromosome wheats the F_1 grains are plump when the 21-chromosome species is female and wrinkled when it is male. In the former case the endosperm of the seed is diploid with respect to the extra 7 *vulgare* chromosomes, and in the latter case haploid.

2. In backcrosses of F_1 with *vulgare* the great majority of seeds are plump when *vulgare* is female, though wrinkled ones occur; in the reciprocal nearly all are wrinkled or badly shrivelled. In backcrosses with emmers also the seeds are better when the pure parent is female.

3. The chromosome numbers of many backcross plants produced by different kinds of seeds (plump, wrinkled, shrivelled, large, small) were determined. This was done for three kinds of F_1 backcrossed with their *vulgare* parent, male and female, and with their emmer parent, male and female. From the number found the number in the endosperm of the seed from which each plant grew was calculated. The number in the endosperm was then compared with the physical condition of the endosperm.

4. The endosperm is plump and large when it contains (a) none or few of the extra 7 *vulgare* chromosomes, (b) 3 sets of 7, complete or nearly so. It is usually plump and small when it contain 2 sets, complete or nearly so. It is wrinkled or shrivelled when (a) it is haploid for all or many of the 7, (b) diploid or triploid for some only. The farther the chromosome

situation departs from the complete absence or complete diploidy or triploidy of the *vulgare* chromosomes, the severer is the shrivelling.

5. Endosperm conditions, depending in this way on chromosome conditions, play a large part in the non-appearance of many types in F_2 and later generations of ordinary crosses. The missing plants would have such chromosome conditions that the endosperm of the seeds from which they would have to develop would be badly shrivelled (or absent).

6. In genetic analysis and economic work on such crosses special care in selection and culture should be given to shrivelled seeds since they represent cytological and genetical types not found among the plump ones.

7. The advantages of using the parent with the larger chromosome number as the female, and of backcrosses are emphasized.

8. Apart from these crosses between *vulgare* and emmer wheats, differences in the success or other results of reciprocal crosses in general may be due in part to endosperm conditions.

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A LETHAL FACTOR IN CREPIS EFFECTIVE ONLY IN AN INTERSPECIFIC HYBRID*

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HISTORICAL INTRODUCTION

BABCOCK and COLLINS (1920, a and b) described hybrids between *Crepis capillaris* (L.) Wallr. (n=3) and *C. tectorum* L. (n=4) which died after developing to the cotyledon stage. Similar results were obtained by NAWASCHIN (1926) but later (1927) he reported a spontaneous *capillaris-tectorum* hybrid which developed normally. He suggested that the phenomenon might be associated with heterozygosity of the parents and later he obtained viable artificial hybrids (unpublished data). *C. capillaris* and *C. tectorum* are taxonomically more closely related than some other species of the genus which produce vigorous interspecific hybrids and it was an interesting result when early attempts to cross them gave inviable seedlings only.

Following the investigations of BABCOCK and COLLINS, Professor BABCOCK'S assistant, Mr. C. W. HANEY, in 1924 again crossed these two species, and out of the five populations which he obtained, four contained 69 hybrids all of which died, but a fifth was made up of 40 hybrids, 20 of which died at the cotyledon stage but 20 matured as large vigorous hybrids. Figure 1 shows the parental species and one of these F_1 hybrids at maturity. The hybrids were intermediate or more like *capillaris* in habit of growth but in most other characters were more like *tectorum*. They were nearly sterile.

In the summer of 1926 the writer was given the material to determine if possible whether there was a factorial basis for the phenomenon just de-

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scribed. The investigation was carried out in the laboratories of the Division of Genetics, UNIVERSITY OF CALIFORNIA. The writer wishes to express her thanks to Mr. C. W. HANEY for his advice and assistance in the culture of plants as well as for the data given above. The plan of investigation was suggested by Professor E. B. BABCOCK and it is a pleasure for the writer to acknowledge her indebtedness to him and to Professor J. L. COLLINS for the material and for helpful advice throughout the course of the work.

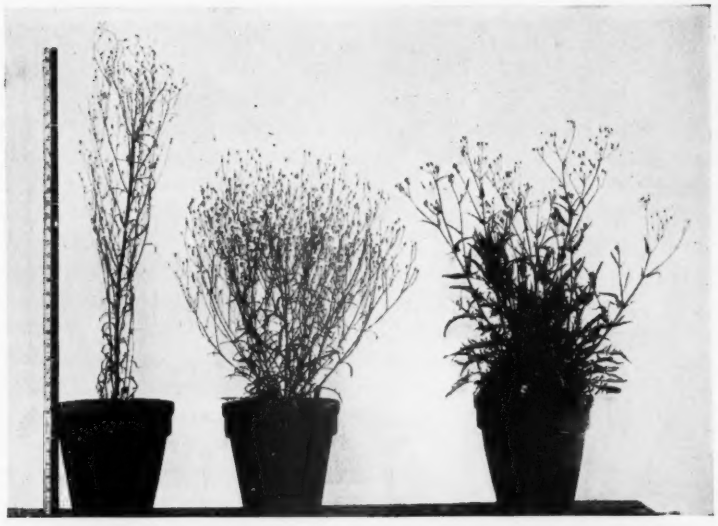

FIGURE 1.—Typical plants of *C. tectorum*, F_1 *C. capillaris-tectorum* and *C. capillaris* at maturity.

MATERIALS AND METHODS

Crepis is a genus of the Cichorieae tribe of the Compositae. *C. capillaris*, outstanding among flowering plants for its small chromosome number, belongs to the same section (Eucrepis) of the genus as *C. tectorum* (BABCOCK and LESLEY 1926) They differ chiefly in leaf shape, habit of growth, flower color, and shape and size of the achenes.

The *capillaris* plant (X.23.1) used in the cross which gave half viable hybrids was a stray plant picked up in the greenhouse by Mr. HANEY and is probably from one of the European strains. The designation X has been kept for the progeny of this plant throughout the investigation. It gave

rise to a particularly vigorous and early-flowering strain, producing under favorable conditions many branches bearing a large number of heads (figure 1). Most plants fruit well in the greenhouse or outdoors. The *tectorum* plant (T.23.1498-4) was one grown from original seed obtained from the COPENHAGEN BOTANICAL GARDEN, and its progeny have been designated by the accession number 1498. It is a vigorous uniform strain and sets fruit fairly well under bags.

The method of pollination used was a modification of that described by COLLINS (1922). The ligules and stamen tubes were removed from the outer row of florets on a head shortly after these florets opened. The unopened florets were removed and the pollen was washed from the styles with a stream of water from a dentist's chip blower. The head was then encased in a light paper bag to dry and to await pollination when the stigmatic branches should be separated. Pollination was usually carried out on the same or the succeeding day by rubbing the stigmas with opened heads of the plant to be used as a male parent. In the first season of crossing (1927) unprotected heads which had opened only the morning they were used were occasionally selected to provide pollen, but experience showed that even in that short time contamination might have taken place and in later investigations only heads protected by bags were used. The pollinated heads were again bagged and removed when the achenes were mature, about three weeks later.

The number of selfed plants obtained by this method of hybridization with *capillaris* as the female parent was small, averaging a little more than 2 percent in 1927 when 34 pure *capillaris* and 1664 F_1 hybrids were obtained. Seventeen of these *capillaris* plants comprised two whole populations. This does not include the four haploid plants of *C. capillaris* which were found in these cultures. A preliminary note on the occurrence of two of them has been published (HOLLINGSHEAD 1928), and it is hoped to report on them in detail in a later paper. The cross was usually made on *capillaris* since it was early learned that the reciprocal (at least the one involving the 1498 strain of *tectorum*) was only rarely successful and that selfed progeny occurred relatively more often. Only six of the *capillaris-tectorum* hybrids recorded in this paper were from achenes produced on *tectorum* plants. BABCOCK and COLLINS (1920 a and b) showed that the reciprocal hybrids obtained in their investigations were inviable and those obtained by the writer have been grouped together in the data given below.

The progeny of the original *tectorum* plant, T.23.1498-4, were from achenes produced on the plant while isolated from other *tectorum* plants in the greenhouse. All other *tectorum* 1498 seed was taken from bagged, or in

one instance, caged plants. The achenes were planted in sterilized sand and the very young seedlings were transplanted to flats or pots.

INVESTIGATIONS INVOLVING THE 1498 STRAIN OF TECTORUM

When the writer took up the work the problem was to determine (1) whether the difference between the normal and abnormal hybrids was due to one or more factors, and (2) if so, from which parent they came. The *capillaris* parent of the hybrid population of which half grew to maturity was also the parent of another population of 57 hybrids all of which died. This indicated that it was perhaps the *tectorum* parent which was responsible for the different behavior of this population. The 1:1 ratio of viable to inviable hybrids suggested that this plant might have been heterozygous for a lethal factor which killed the interspecific hybrid into which it entered. To test this hypothesis it was planned to grow a progeny of this *tectorum* plant and to cross each individual of it to *capillaris*. If the *tectorum* parent had been heterozygous for an interspecific lethal the progeny should be segregating for it and the genotypic constitution of each plant should be indicated by the kind of hybrids it produced when crossed to *capillaris*.

When the investigation was begun in August 1926 there were growing a number of *tectorum* plants, T.26.1498-1 to 30, progeny of this original *tectorum* T.23.1498-4. Most of them were dead or dying but a few, after being cut back, produced some more heads. They were used chiefly as male parents in crosses with *capillaris*, and some hybrid achenes were obtained. Three strains of *capillaris* were used but most of the crossing involved a plant of the X strain. The possibility that the *capillaris* parent might have something to do with the inviability of the hybrid offspring was constantly in mind throughout the early part of the work and this was the chief reason why the X strain, the one involved in the cross which first gave viable hybrids, was the one chiefly used. Evidence to be given later indicated that the *capillaris* plants used had no effect on the viability of the hybrid offspring and in the tables the hybrids are grouped without regard to the *capillaris* parent.

The achenes obtained from these first crosses were planted in December, 1926, but unfortunately the young plants were attacked by red spiders and in a few cases it was difficult to tell whether the seedlings died because of the spiders or whether they were exhibiting the phenomenon which had come to be known as "dying at the cotyledon stage." The results of this preliminary work are given in the first four lines of table 1. It was suspected that those hybrids in the progenies of T.26.1498-26 and T.26.1498-

24 which died owed their death to the spider attack and this was substantiated by investigations to be described later.

Hoping to obtain plants for crossing during the winter months another lot of achenes from the original *tectorum* T.23.1498-4 was planted in August, 1926 and grown in the greenhouse under the numbers T.26.1498-60 to -95 and at the same time some *capillaris* strains were planted. The *tectorum* plants which first sent up flower stalks were attacked by spiders and did not prove to be very vigorous, while the *capillaris* had not yet bloomed. It was decided that conditions were unfavorable for hybridization during the winter and the plants were put outside in the hope of keeping them till spring. Most of them died but a few bloomed late enough in the spring to be available for crossing. The last of the achenes from the original *tectorum* plant were sown in February, 1927, and the plants were grown under the numbers T.27.1498-21 to 40. The population was a vigorous rather uniform one with no outward sign of a lethal factor present in any of the plants.

This provided in June, 1927, a group of *tectorum* plants, progeny of the original T.23.1498-4, each of which was crossed to *capillaris*. An effort was made to obtain at least twenty hybrid achenes from each *tectorum* parent to ensure a progeny large enough to make reasonably valid the indicated parental genetic constitution. As table 1 shows, many more than twenty were obtained in most cases, but low germination in some instances greatly reduced the number of hybrids obtained below the number expected. Variation in percentage of germination characterized the whole investigation and in a few cases the number of hybrids obtained was too small to indicate the parental constitution and these have been omitted in this table.

The results obtained were in agreement with the hypothesis that this population of *tectorum* plants was segregating for a lethal factor effective in an interspecific hybrid but without effect on *tectorum*. The results are summarized in table 1. The hybrids are grouped with respect to the *tectorum* parent and each group will be described as a "population" although in most cases the hybrids in a group were obtained from two or more *capillaris* plants. The footnotes to this table and to those which follow explain why a few hybrids were not classified. The one case here attributed to experimental error is probably due to contamination of heads used for pollination. As the table shows, some *tectorum* plants gave hybrids, all of which lived, indicating no lethal in the parent ($L\ L$). A larger group gave hybrids, part of which lived and part of which died, indicating a parent heterozygous for the lethal ($L\ l$). Some gave hybrids, all of which died,

indicating parents homozygous for the lethal (*l l*). The letters *l*, for the lethal, and *L*, for the allelomorph, have been assigned arbitrarily and do not indicate any dominant or recessive relationships.

TABLE 1

F_1 hybrids obtained by crossing progeny of original C. tectorum plant T.23.1498–4 to capillaris.

tectorum PARENT	F_1 ACHENES	GERMINATED	UNCLASSIFIED[3]	LIVED	DIED	GENOTYPE OF *tectorum* PARENT
T.26.1498–26	26	21		18	3	*L L*[1]
" " –24	32	13		12	1	*L L*[1]
" " –19	14	9		0	9	*l l*
" " –23	18	7		0	7	*l l*
T.26.1498–63	98	58		0	58	*l l*
" " –68	39	25		0	25	*l l*
" " –77	37	15		15	0	*L L*
" " –78	24	15		15	0	*L L*
" " –82	43	15		15	0	*L L*
" " –84	40	18	1	17	0	*L L*
" " –86	9	7		5	2	*L l*
" " –88	48	40		0	40	*l l*
" " –91	26	12	1	11	0	*L L*
T.27.1498–21	44	39	1	16	22	*L l*
" " –22	45	32		32	0	*L L*
" " –23	47	22		18	4	*L l*
" " –24	29	11		11	0	*L L*
" " –26	27	13		0	13	*l l*
" " –27	54	45		20	25	*L l*
" " –28	26	18		9	9	*L l*
" " –29	23	23		10	13	*L l*
" " –30	32	16	1	7	8	*L l*
" " –31	71	47	1	0	46	*l l*
" " –32	28	21		7	14	*L l*
" " –34	36	30		12	18	*L l*
" " –35	34	22		12	10	*L l*
" " –36	43	23		0	23	*l l*
" " –37	51	30		15	15	*L l*
" " –38	24	14		5	9	*L l*
" " –39	23	8		4	4	*L l*
" " –40	32	12		11	1[2]	*L L*

LL : *Ll* : *ll*
10: 13: 8

[1] Red spiders attacked the young plants. Progeny tests (see tables 2 and 3) showed the *tectorum* parents were free from the lethal.

[2] Probably due to experimental error, the chances of obtaining such a ratio from a heterozygous parent being 3 in 1000.

[3] Did not survive transplanting.

As noted above two of the *tectorum* plants tested in 1926 gave hybrids most of which lived, and presumably those which died owed their death to red spiders. In order to prove that these two plants were really free from the lethal, progenies of each of them were grown in 1927 and crossed to *capillaris*. The results are summarized in tables 2 and 3. Nine plants of the progeny of T.26.1498-26 gave 148 hybrids, all living, indicating they were free from the lethal. The chance of obtaining nine homozygous plants from a heterozygous parent is only 1 in 512 so that it is most probable that the *tectorum* grandparent of these hybrids, T.26.1498-26, was free from the lethal and it has been so classified. Fifteen plants of the progeny of T.26.1498-24 gave 292 hybrids of which only one died. It was in a population of 25 hybrids and has been attributed to experimental error. A progeny test of its parent, T.27.1498-11 (table 4), showed no evidence of a lethal. These results indicate that the *tectorum* grandparent, T.26.1498-24, was also free from the lethal.

TABLE 2

F_1 hybrids obtained by crossing progeny of T.26.1498–26 to capillaris.

tectorum PARENT	F_1 ACHENES	GERMINATED	UNCLASSIFIED	LIVED	DIED	GENOTYPE OF *tectorum* PARENT
T.27.1498–41	50	23		23		*L L*
" " –42	25	17		17		*L L*
" " –43	21	17		17		*L L*
" " –45	47	27		27		*L L*
" " –46	27	7		7		*L L*
" " –47	14	6		6		*L L*
" " –48	33	28		28		*L L*
" " –49	39	15		15		*L L*
" " –50	20	8		8		*L L*
Total	276	148		148		

The genotypes of the progeny of the original *tectorum* plant having been established the distribution proved to be 10 *L L* : 13 *L l* : 8 *l l*. The expected distribution for a single factor difference would be 7.75 *L L* : 15.5 *L l* : 7.75 *l l*. Such a difference between the actual and the expected as tested by the χ^2 method would occur six times in ten by random sampling and the distribution can therefore be accepted as agreeing with the theoretical expectation.

In order to further establish the conclusion that we were dealing with a simple lethal factor which did not affect the *tectorum* plant bearing it but which caused the death of the interspecific hybrid into which it entered

another season's work was planned. The genotypes of the various *tectorum* plants having been determined and achenes having been obtained from

TABLE 3

F_1 hybrids obtained by crossing progeny of T.26.1498–24 to capillaris.

tectorum PARENT	F_1 ACHENES	GERMINATED	UNCLASSIFIED[1]	LIVED	DIED	GENOTYPE OF *tectorum* PARENT
T.27.1498– 2	23	9		9		*L L*
" " – 3	27	16		16		*L L*
" " – 4	42	14	1[1]	13		*L L*
" " – 5	47	27		27		*L L*
" " – 6	23	22		22		*L L*
" " – 8	25	10		10		*L L*
" " – 9	22	12		12		*L L*
" " –10	35	25		25		*L L*
" " –11	37	25		24	1[2]	*L L*
" " –12	59	28	1[3]	27		*L L*
" " –14	20	18		18		*L L*
" " –15	37	17		17		*L L*
" " –16	37	26		26		*L L*
" " –18	32	18		18		*L L*
" " –20	45	27		27		*L L*
Total	511	294	2	291	1	

[1] Abnormal growing point and thick leathery leaves. Died very early.
[2] Doubtless due to experimental error.
[3] Did not survive transplanting.

most of them, two of each genotype were chosen to provide progeny for further work. The six plants and their progenies were as follows:

Genotype	*Plant*	*Progeny*
L L	T.27.1498–11	28H.T.1498D–1 to–24
	T.27.1498–15	28H.T.1498E–1 to–16
L l	T.27.1498–30	28H.T.1498F–1 to–24
	T.27.1498–34	28H.T.1498G–1 to–32
l l	T.27.1498–31	28H.T.1498H–1 to–24
	T.27.1498–36	28H.T.1498 I–1 to–16

As many plants as possible of each progeny were crossed to *capillaris* in the summer of 1928. The achenes obtained were sown in the fall of 1928 and the results obtained are summarized in tables 4 to 9.

The progeny of the first *L L* plant, T.27.1498-11, gave 22 hybrid populations containing 249 plants all developing normally (table 4). Seventeen

of these populations contained at least six hybrids, the smallest number from which it is possible to determine the parental genotype with any degree of certainty (the chance of obtaining six viable hybrids from a heterozygous parent is 1 in 64), and these were classified *L L*, while the nature of the other five populations with fewer hybrids was in agreement with the expectation that all the *tectorum* plants of this group were *L L*. The

TABLE 4

F_1 hybrids obtained by crossing progeny of T.27.1498–11 (L L) to capillaris.

tectorum PARENT	F_1 ACHENES	GERMINATED	UNCLASSIFIED	LIVED	DIED	GENOTYPE OF *tectorum* PARENT
28H.T.1498D– 1	15	6		6		*L L*
" " – 2	27	22		22		*L L*
" " – 3	2	2		2		
" " – 4	15	13		13		*L L*
" " – 5	14	12		12		*L L*
" " – 6	27	10		10		*L L*
" " – 8	18	16		16		*L L*
" " – 9	10	10		10		*L L*
" " –10	10	7		7		*L L*
" " –12	11	9		9		*L L*
" " –13	29	13		13		*L L*
" " –14	16	5		5		
" " –15	25	24		24		*L L*
" " –16	26	17		17		*L L*
" " –17	19	11		11		*L L*
" " –18	9	8		8		*L L*
" " –19	8	5		5		
" " –20	20	14		14		*L L*
" " –21	23	21		21		*L L*
" " –22	9	5		5		
" " –23	21	15		15		*L L*
" " –24	5	4		4		
Total	359	249		249		

progeny of the second *L L* plant, T.27.1498-15, gave 10 hybrid populations including in all 149 hybrids, all normal (table 5). Eight of these populations contained enough hybrids to enable the parents to be classified as normal. This confirms the expectation that plants genetically *L L* give only *L L* progeny and that races were isolated free from the lethal.

The progeny of T. 27.1498-30 (*L l*) were segregating for the lethal as shown by the occurrence again of hybrid populations of three types: all living; part living, part dying; all dying (table 6). The distribution was 6 *L L* : 11 *L l* : 7 *l l* a close approximation to the expected 6 *L L*: 12 *L l*

TABLE 5

F_1 hybrids obtained by crossing progeny of T.27.1498–15 (L L) to capillaris.

tectorum PARENT	F_1 ACHENES	GERMINATED	UNCLASSIFIED	LIVED	DIED	GENOTYPE OF *tectorum* PARENT
28H.T.1498E– 2	32	26		26		*L L*
" " – 4	19	18		18		*L L*
" " – 5	2	2		2		
" " – 8	53	46		46		*L L*
" " –10	10	5		5		
" " –11	15	12		12		*L L*
" " –12	11	8		8		*L L*
" " –14	28	17		17		*L L*
" " –15	24	9		9		*L L*
" " –16	6	6		6		*L L*
Total	200	149		149		

TABLE 6

F_1 hybrids obtained by crossing progeny of T.27.1498–30 (L l) to capillaris.

tectorum PARENT	F_1 ACHENES	GERMINATED	UNCLASSIFIED[1]	LIVED	DIED	GENOTYPE OF *tectorum* PARENT
28H.T.1498F– 1	19	6		6	0	*L L*
" " – 2	24	11		4	7	*L l*
" " – 3	23	16		10	6	*L l*
" " – 4	27	12		9	3	*L l*
" " – 5	22	14		6	8	*L l*
" " – 6	21	15		0	15	*l l*
" " – 7	28	13		0	13	*l l*
" " – 8	17	14		14	0	*L L*
" " – 9	14	9		6	3	*L l*
" " –10	26	13		0	13	*l l*
" " –11	19	14		4	10	*L l*
" " –12	36	24		11	13	*L l*
" " –13	33	15		15	0	*L L*
" " –14	17	6		6	0	*L L*
" " –15	27	24		13	11	*L l*
" " –16	31	10		0	10	*l l*
" " –17		7		2	5	*L l*
" " –18		14		0	14	*l l*
" " –19		9	1	0	8	*l l*
" " –20	28	13		9	4	*L l*
" " –21	22	18		18	0	*L L*
" " –22	22	11		11	0	*L L*
" " –23	24	7		5	2	*L l*
" " –24	21	11		0	11	*l l*

L L:*L l*:*l l*
6: 11: 7

[1] Did not survive transplanting.

: 6 *l l*. Similarly the progeny of T.27.1498-34 (*L l*) showed a distribution 4 *L L* : 19 *L l* : 4 *l l* (table 7). Such a deviation from the expected 6.75 *L L* : 13.5 *L l* : 6.75 *l l* would occur 11 times in 100 by random sampling and the distribution is therefore in agreement with expectation.

TABLE 7

F_1 hybrids obtained by crossing progeny of T.27.1498–34 (L l) to capillaris.

tectorum PARENT	F_1 ACHENES	GERMINATED	UNCLASSIFIED	LIVED	DIED	GENOTYPE OF *tectorum* PARENT
28H.T.1498G– 1	20	12		7	5	*L l*
" " – 2	26	20		0	20	*l l*
" " – 3	23	5		2	3	*L l*
" " – 5	10	8		3	5	*L l*
" " – 7	29	17		17	0	*L L*
" " – 9	25	7		3	4	*L l*
" " –10	23	5		2	3	*L l*
" " –11	18	14		9	5	*L l*
" " –12	15	13		0	13	*l l*
" " –13	31	17		9	8	*L l*
" " –14	19	4		3	1	*L l*
" " –15	43	18		9	9	*L l*
" " –16	29	6		6	0	*L L*
" " –17	45	26		26	0	*L L*
" " –18	43	13		7	6	*L l*
" " –19	33	32	1[1]	14	17	*L l*
" " –20	35	13		7	6	*L l*
" " –21	29	19		9	10	*L l*
" " –22	13	12		6	6	*L l*
" " –23	20	3		1	2	*L l*
" " –24	19	8		4	4	*L l*
" " –25	21	16		0	16	*l l*
" " –26	30	13		7	6	*L l*
" " –27	30	21	1[2]	20	0	*L L*
" " –28	21	16		5	11	*L l*
" " –30	20	11		9	2	*L l*
" " –32	21	7		0	7	*l l*
				LL:	*L l*:	*l l*
				4:	19:	4

[1] Did not survive transplanting.

[2] Abnormal, died early.

The progeny of T.27.1498-31 (*l l*) gave 19 hybrid populations containing 157 plants all of which were inviable (table 8). Eleven of these contained enough hybrids to enable the parents to be classified as *l l*. The progeny of T.27.1498-36 (*l l*) gave 14 hybrid populations containing 185 seedlings, all of which died (table 9). Twelve of these contained enough hybrids to enable the parents to be classified as *l l*. Here races were established homozygous for the lethal.

TABLE 8

F_1 hybrids obtained by crossing progeny of T.27.1498–31 (l l) to capillaris.

tectorum PARENT	F_1 ACHENES	GERMINATED	UNCLASSIFIED	LIVED	DIED	GENOTYPE OF *tectorum* PARENT
28H.T.1498H– 2	17	12			12	*l l*
" " – 3	10	7			7	*l l*
" " – 4	17	13			13	*l l*
" " – 5	5	4			4	
" " – 6	9	9			9	*l l*
" " – 7	17	13			13	*l l*
" " – 8	12	11			11	*l l*
" " – 9	14	13			13	*l l*
" " –11	7	3			3	
" " –12	13	11			11	*l l*
" " –13	23	9			9	*l l*
" " –14	4	1			1	
" " –15	27	19			19	*l l*
" " –16	27	1			1	
" " –17	22	18			18	*l l*
" " –19	2	2			2	
" " –21	7	5			5	
" " –23	2	1			1	
" " –24	7	5			5	
Total	242	157			157	

TABLE 9

F_1 hybrids obtained by crossing progeny of T.27.1498–36 (l l) to capillaris.

tectorum PARENT	F_1 ACHENES	GERMINATED	UNCLASSIFIED[1]	LIVED	DIED	GENOTYPE OF *tectorum* PARENT
28H.T.1498I– 1	21	8			8	*l l*
" " – 2	16	3			3	
" " – 3	37	5			5	
" " – 4	22	14			14	*l l*
" " – 5	22	7			7	*l l*
" " – 6	6	6			6	*l l*
" " – 7	29	16			16	*l l*
" " – 8	27	15			15	*l l*
" " –10	29	26			26	*l l*
" " –11	26	11			11	*l l*
" " –13	31	27			27	*l l*
" " –14	27	25			25	*l l*
" " –15	20	16	1		15	*l l*
" " –16	8	7			7	*l l*
Total	321	186	1		185	

[1] Did not survive transplanting.

Figures 2, 3, and 4 show three flats of young hybrids whose *tectorum* parents were the progeny of plants of the three genotypes. Figure 2 shows hybrids whose various *tectorum* parents were the progeny of *L L* plants—the hybrids are all developing normally. Figure 3 shows hybrids whose *tectorum* parents were the progeny of *L l* plants. Each kind of hybrid population obtained from a segregating group of *tectorum* plants is represented: all living; part living; part living, part dying; all dying.

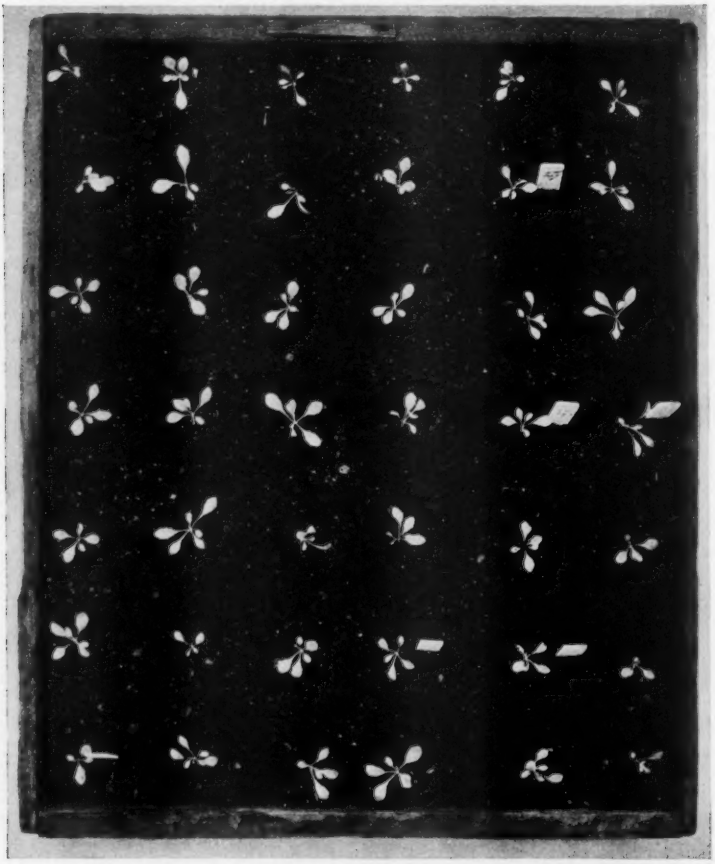

FIGURE 2.—*C. capillaris-tectorum* hybrids whose *tectorum* parents were *LL*. A label marks the first plant of a population numbering from below in each row.

Figure 4 shows hybrids whose *tectorum* parents were the progeny of *l l* plants—all are dying at the cotyledon stage.

Table 10 summarizes the distribution of the three genotypes in the original segregating population and in the two just described. The agreement between the total actual and the expected is very close.

It was stated earlier that the *capillaris* parent of a hybrid, so far as has been determined, had no effect on the expression of the lethal. Table 11 shows the nature of eight populations of hybrids classified with respect

FIGURE 3.—*C. capillaris-tectorum* hybrids whose *tectorum* parents were *Ll*.

TABLE 10

Distribution of tectorum plants in the three segregating populations investigated.

HETEROZYGOUS *tectorum* PARENT	SEGREGATING POPULATION	*L L*	*L l*	*l l*
T.23.1498–4	(T.26.1498–1 to 91 (T.27.1498–21 to 40	10	13	8
T.27.1498–30	28H.T.1498F–1 to 24	6	11	7
T.27.1498–34	28H.T.1498G–1 to 32	4	19	4
	Total	20	43	19
	Expected	*20.5*	*41*	*20.5*

FIGURE 4.—*C. capillaris-tectorum* hybrids whose *tectorum* parents were *ll*.

to their *capillaris* parents. An inspection of the table shows that the various hybrid groups obtained from different *capillaris* parents and the

TABLE 11

Eight hybrid populations classified with respect to their capillaris parents.

tectorum PARENT	*capillaris* PARENT	HYBRIDS LIVED	HYBRIDS DIED
T.26.1648–3	Crane 26–1	2	0
	X 26–2	8	0
T.26.1498–68	D 26–8	0	1
	X 27–3	0	2
	X 27H. 8–3	0	7
	X 27H.21–4	0	15
T.26.1498–91	D 26–8	2	0
	X 27–4	2	0
	X 27H.21–4	7	0
T.27.1498–22	Crane 27H. 9–6	6	0
	X 27H.20–6	26	0
T.27.1498–36	Crane 27H. 9–6	0	7
	X 27–4	0	3
	X 27H.20–6	0	3
	X 27H. 8–11	0	10
T.27.1498–37	Crane 27H. 9–6	4	8
	X 27H. 8–5	8	3
	X 27H. 8–3	3	4
T.27.1498	D 26–2	0	1
	X 27–3	0	5
	X 27H.20–6	0	11
	X 27H.21–4	0	29
T.27.1498–34	D 26–3	2	0
	D 26–8	0	1
	X 27H.20–6	6	7
	X 27H. 8–7	4	8
	X 27–3	0	2

same *tectorum* parent behave similarly with respect to the lethal. There were three strains of *capillaris* involved. The origin of the X strain has been described. *Capillaris* D is an inbred strain from material collected for Professor J. L. COLLINS near Eureka, California. The Crane strain is from a derivative of a cross between D and another inbred strain from the same

locality. The Crane and D strains have less vigorous habit and mature later than X and the Crane strain was segregating for the "chlorina' character described by COLLINS (1924).

It was expected on the basis of a simple lethal factor that the hybrid progeny of heterozygous (*L l*) *tectorum* plants would give a 1:1 ratio of living to dying individuals. In no case, except the one attributed to experimental error (progeny of T.27.1498-40, table 1), was a distribution found which could not be harmonized with such a ratio. In many cases a good 1:1 ratio was obtained in a single population; in many others the number was too small to give any significance to the distribution. On totalling all the populations from *L l tectorum* parents the distribution was found to be 346 living to 339 dying, a good 1:1 ratio.

The percentage of germination of the hybrid achenes is of some interest. As may be seen from the tables wide variations occurred. Table 12 shows,

TABLE 12

Percentage of germination of capillaris-tectorum (1498) hybrids grown in the spring and fall of 1928.

	SPRING 1928			FALL 1928			TOTAL		
	ACHENES	GERMINATED	PERCENTAGE	ACHENES	GERMINATED	PERCENTAGE	ACHENES	GERMINATED	PERCENTAGE
Populations from *L L* parents	1063	572	53.81	822	538	65.45	1885	1110	58.88
Populations from *l l* parents	326	206	63.19	773	461	59.64	1099	667	60.69
Populations from *L l* parents	431	295	68.44	729	374	51.30	1160	669	57.67
Total	1820	1073	58.96	2324	1373	59.08	4144	2446	59.02

however, that for large numbers the percentage is rather constant. The data include all the hybrids between *tectorum* 1498 and *capillaris* made in the summers of 1927 and 1928 and grown in the spring and fall of 1928 respectively (omitting 3 populations in table 6 whose seed pots were upset and an unknown number of achenes lost). The average percentage amounts to only 59.02 percent. There is no consistent difference either between the two crops or between hybrid progenies of parents of different genotypes. These data show that there is no differential germination of hybrids with respect to the lethal and this conclusion is confirmed by the 1:1 ratio of living to dying hybrids from heterozygous parents.

The inviable hybrids obtained by BABCOCK and COLLINS in only a few cases developed tiny first leaves; usually development ceased before any trace of leaves was to be seen. This condition characterized most of the inviable hybrids which were obtained in this investigation but some developed further. Figure 5 is a picture of a normal hybrid and six inviable ones

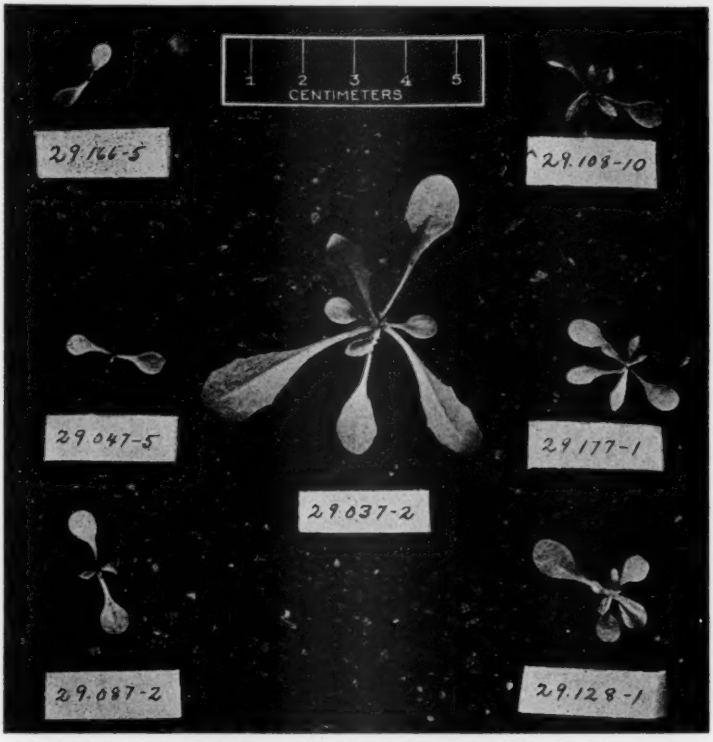

FIGURE 5.—A viable F_1 *C capillaris-tectorum* F_1 hybrid (centre) and six inviable ones chosen to illustrate various degrees of development to which inviable hybrids attained.

transplanted into a small box from their positions in the flats. The inviable ones were chosen to illustrate the various degrees of development to which the hybrids attained. Figure 6 is a picture of the same box of hybrids a month later. In the interval the first four inviable ones died without developing further, but two continued to produce small granular leaves, darker

green in color and coarser in texture than normal leaves. They were showing signs of coming death when the picture was taken. In only a few cases did the inviable hybrids develop to a stage comparable with these last two

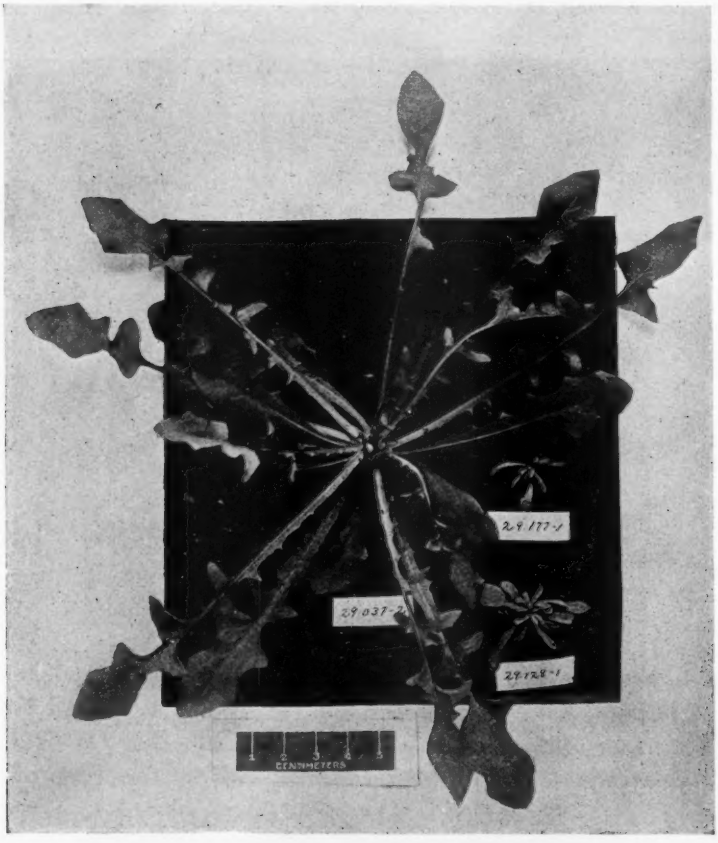

FIGURE 6.—The same box of hybrids a month later.

Three hybrids which had ceased development at the cotyledon stage and two which were obviously abnormal but which had produced a number of leaves were fixed and sectioned for anatomical examination. Normally developing hybrid seedlings of the same age were examined for

comparison. The most striking feature of the inviable hybrids was the occurrence of masses of disintegrating cells which appeared like black slime. This condition was especially characteristic of those seedlings which had ceased development early, being very prevalent in the region just back of the plumule and around the vascular system of the stem and cotyledons. While it was much less prevalent in abnormal hybrids which produced a number of leaves it was more noticeable in them than in normal hybrid seedlings in which there was little or no disintegration. All of the inviable hybrids showed disorganized parenchymatous tissue, the cells being irregular in arrangement and exhibiting striking variations in size and shape which in some cases were probably due to the collapse of intervening cell walls. The plumules of the inviable hybrids which produced no leaves showed several buds in various stages of early development, most of the tissues being still meristematic. The beginnings of differentiation were to be seen in the larger size and vacuolated appearance of some of the cells and in the occurrence of strands of elongated meristematic cells extending upwards through the undeveloped buds. In one plant tracheids were observed extending to within two cell layers of the surface of a bud. These tracheids were much shorter than the recently differentiated tracheids of normal hybrids. The occurrence of tracheids in the meristematic tissue of a very small developing bud is quite abnormal.

The inviable hybrids which developed tiny leaves were still growing when fixed and showed a normal growing point and buds in various stages of development. The shape of the young plant was broad and flat in contrast with the narrower and more upstanding normal hybrid. Irregularity in arrangement and size of the cells of the parenchymatous tissue and the occurrence of small areas of dead cells distinguished them from normal hybrids. Particularly interesting was the occurrence of groups of small cells, sometimes meristematic, which in shape, size, and position resembled axillary buds. In no case did they appear to be developing into leaves. No such structures were seen in normal hybrids.

BABCOCK and COLLINS (1920a) described patches of black slime and irregularities in parenchymatous tissue in their inviable hybrids. Patches of embryonic tissue and of tracheary cells were distributed here and there among the larger vegetative cells. While this latter condition was not observed in these hybrids it could hardly be expected that disorganization causing cessation of development would manifest itself always in the same way and it has been shown above that the various inviable hybrids examined differed noticeably in the degree and kind of irregularities.

INVESTIGATIONS INVOLVING OTHER STRAINS OF TECTORUM

Concurrently with the investigations just described crosses were made in an attempt to discover how widely distributed was the supposed lethal factor in *tectorum*. Achenes from a number of sources obtained over a period of years were sown and as many plants as possible were tested by crossing to *capillaris*. Table 13 gives the sources of the *tectorum* plants used, and the number and kinds of hybrids obtained from them.

TABLE 13

F_1 hybrids obtained by crossing tectorum plants of various origins to capillaris.

ORIGIN OF *tectorum* PARENT	*tectorum* PARENT	F_1 ACHENES	GERMINATED	LIVED	DIED
Original seed from Stockholm Botanical Garden	T.27.1066– 1	13	11	0	11
	" " – 2	20	10	0	10
	" " – 3	24	9	0	9
	" " – 4	19	10	0	10
Ditto	T.27.1067– 1	21	10	0	10
Original seed? Chelsea Botanical Garden	T.27.1503– 1	15	13	0	13
	" " – 2	14	13	0	13
	" " – 5	36	22	0	22
Copenhagen Botanical Garden through Nawaschin.	T.27.1622– 2	8	8	0	8
	" " – 3	11	4	4	0
Ditto	T.27.1644– 1	12	6	0	6
	" " – 2	22	15	0	15
	" " – 3	7	2	1	1
	" " – 4	14	10	6	4
Original seed from near Tomsk, Siberia.	T.26.1648– 3	12	10	10	0
	T.27.1648– 4	12	12	6	6
	" " – 9	27	21	12	9
Original seed from near Kiev.	T.27.1689– 4	32	23	0	23
Progeny of T.26.1700–6 (open), original seed from Insula Oland, Sweden.	T.27.1700– 1a	23	3	3	0
	" " – 3a	22	18	9	9
	" " – 5a	31	18	18	0
	" " – 6a	13	3	3	0
	" " – 8a	10	6	0	6
	" " –12a	10	10	6	4
Original seed from Upsala Botanical Garden through Berlin.	T.27.1719– 1	26	15	0	15
Original seed from Stockholm Botanical Garden through Berlin.	T.27.1726– 1	22	15	0	15

TABLE 13 (*continued*)

ORIGIN OF *tectorum* PARENT	*tectorum* PARENT	F_1 ACHENES	GERMINATED	LIVED	DIED
Original seed from Stockholm Botanical Garden through Berlin.	T.27.1757– 3	11	4	0	4
Original seed from Copenhagen Botanical Garden through Berlin.	T.27.1758– 1	15	7	3	4
	" " – 2	10	6	0	6
	" " – 5	5	1	1	0
Original seed, unknown, through Berlin.	T.27.1760– 1	23	10	0	10
	" " – 2	28	8	0	8
	" " – 3	12	6	0	6
	" " – 4	29	12	0	12
Original seed from Stockholm Botanical Garden.	T.27.1883– 1	31	14	0	14
	" " – 2	11	7	0	7
	" " – 3	19	6	0	6
	" " – 4	28	12	0	12
	" " – 5	16	11	0	11
	" " – 6	9	3	0	3
	" " – 7	9	6	0	6
	" " – 8	20	15	0	15
	" " – 9	7	4	0	4
	" " –10	15	9	0	9

Most of the *tectorum* plants tested produced only inviable hybrids and would be classified as homozygous for the lethal. Two accessions, similar in appearance, obtained from Doctor NAWASCHIN and originally from COPENHAGEN BOTANICAL GARDEN were segregating for the lethal. Two plants of the 1648 strain from Tomsk, Siberia, were heterozygous. The population, T.27.1700-a, appeared to be segregating for the lethal, but since its parent, T.26.1700-6, was open-pollinated and the strain is highly self-sterile the lethal may have been introduced from another strain. A strain from the COPENHAGEN BOTANICAL GARDEN contained both the lethal and its allelomorph. From these results it may be seen that the plants to which the lethal can be traced definitely came either from the COPENHAGEN BOTANICAL GARDEN and are probably of Western European origin or from Tomsk in Central Siberia.

INVESTIGATIONS INVOLVING HYBRIDS BETWEEN TECTORUM AND OTHER SPECIES

These investigations were designed to determine whether the lethal which killed *capillaris-tectorum* F_1 hybrids would also be effective in hybrids between *tectorum* and other Crepis species. Table 14 shows the crosses

made and the results obtained. Part of the data on *tectorum-leontodontoides* crosses was kindly supplied by Miss PRISCILLA AVERY who was working with this cross.

TABLE 14

F_1 hybrids between tectorum and other species to show the effect of the lethal.

tectorum PARENT	F_1 ACHENES	GERMINATED	UNCLASSIFIED	LIVED	DIED
28H.T.1498E–4 (*L L*)×bursifolia	4	2	..	2	0
28H.T.1498E–8(*L L*)×bursifolia	6	3	..	3	0
28H.T.1498I–2 (*l l*)×bursifolia	16	3	..	0	3
T.27.1498–22 (*L L*)×leontodontoides	7	4	..	4	0
T.27.1498–23 (*L l*)×leontodontoides	15	10	1[1]	6	3
28H.T.1498I–2 (*l l*)×leontodontoides	28	13	..	0	13
28H.T.1498H–15 (*l l*)×leontodontoides	4	4	..	0	4
T.27.1498–9 (*L L*)×setosa	13	10	..	10	0
T.27.1498–18 (*L L*)×setosa	22	10	..	10	0
T.27.1498–29 (*L l*)×setosa	9	7	..	7	0
28H.T.1498D–18 (*L L*)×taraxacifolia	3	2	..	2	0
28H.T.1498E–8 (*L L*)×taraxacifolia	1	1	..	1	0
28H.T.1498E–14 (*L L*)×taraxacifolia	4	4	..	4	0
28H.T.1498F–4 (*L l*)×taraxacifolia	9	8	..	8	0
T.27.1498–23 (*L l*)×taraxacifolia	6	3	..	3	0
28H.T.1498H–2 (*l l*)×taraxacifolia	3	2	..	2	0
28H.T.1498H–9 (*l l*)×taraxacifolia	3	2	..	2	0
28H.T.1498H–19 (*l l*)×taraxacifolia	2	2	..	1	1

[1] Died early.

The table shows that both viable and inviable hybrids resulted from *tectorum-bursifolia* and *tectorum-leontodontoides* crosses and that they occurred in the populations and in the proportions expected if the lethal were effective. The data on *tectorum-setosa* are not quite conclusive but indicate that the factor is ineffective here since seven viable hybrids were obtained from a *tectorum* plant heterozygous for the lethal and the chance of obtaining such a distribution if the lethal were effective is only 1 in 128.

The data on *tectorum-taraxacifolia* indicate the general ineffectiveness of the lethal in these hybrids. A heterozygous *tectorum* gave eight hybrids, all viable, and the chance of this occurring if the lethal were effective is 1 in 256. Four viable hybrids were obtained from *tectorum* parents homozygous for the lethal but from another one, one viable and one typically inviable hybrid were obtained. Whether the lethal occasionally manifests itself or whether this plant died from other causes has not been determined.

DISCUSSION

This is the first reported instance, so far as the writer knows, of a lethal factor which is effective only in an interspecific hybrid. The phenomenon described by SAX (1921) in hybrids between varieties of wheat resembles the one described here more closely than any other with which the writer is familiar. He describes hybrids between Bluestem and Amby, varieties of *Triticum vulgare*, which produced abnormal hybrids while these varieties crossed with other members of the *vulgare* group gave F_1 plants, fully fertile. The abnormal hybrids between these varieties "appeared to develop normally until they reached a height of 15 to 20 cm. They then ceased to increase in height although they were vigorous and stooled excessively. Often as many as 40 or 50 primary culms were formed but in no case did a true culm develop. The plants were observed for five months but did not develop further." In this case the cross was only a varietal one and the plants in question developed further than the inviable interspecific hybrids in this study. SAX suggested that the case could be analyzed by crossing the F_1 of Amby × Marquis with Bluestem. If a factor similar to the lethal in *Crepis tectorum* were involved, either this cross or the F_1 of Bluestem × Marquis crossed with Amby should reveal it.

In Nicotiana it has been shown by CLAUSEN (BABCOCK and CLAUSEN 1927) that different varieties of a species may give different results with respect to the vigor of hybrids with another species. A series of varieties of *N. Tabacum* were crossed with two varieties of *N. glauca*, one derived from a California source, the other from Argentine. "With the California variety of *N. glauca* the hybrids were uniformly weak with yellowish leaves short internodes, and a total growth not much greater than 2 feet. With the Argentine variety they were exceptionally vigorous with large dark leaves, long internodes and a height ranging from 6 to 12 feet according to the variety of *N. Tabacum* used." It would be of interest to know whether this difference in the F_1 hybrids could be ascribed to simple genetic differences between the varieties of *N. glauca*. Such results warn the investigator against drawing conclusions concerning taxonomic relationship from the viability of interspecific hybrids.

From an evolutionary standpoint there is no evidence as to whether this Crepis lethal or its allelomorph is the older. Both were found in Western European and Central Siberian strains, indicating a wide range for each. Since the lethal has no effect on the parental species, and since normal F_1 interspecific hybrids are almost sterile it is difficult to see how it has had any part in the later evolutionary development of the species in-

vestigated. If the lethal is the older its present existence may be a relic from a time when the species concerned were in the process of differentiation and it may have played a rôle in the maintenance of the ancestral stocks.

The nature of the causes of interspecific incompatibility has long been a matter of considerable interest and speculation. Genetic investigations are slowly bringing to light conditions which may constitute these causes. That crossability depends upon the particular parental variety used has been shown by BACKHOUSE (1916) and by GAINES and STEVENSON (1922) in wheat-rye crosses and later investigators (THOMPSON 1926, LEIGHTY and SANDO 1928) have confirmed this knowledge. The investigations of BACKHOUSE showed that the difference between the varieties of wheat in their ability to cross readily with rye might rest on a factor basis. Here the incompatibility between the parental species manifests itself in failure of fertilization or possibly early development but when that has been accomplished the hybrid grows vigorously. The existence of the lethal factor which operates to kill an interspecific hybrid at an early stage in development and the demonstration of its heritability in a simple Mendelian fashion is a point of importance in this connection.

As was pointed out above the designations *l* and *L* for the lethal and its allelomorph are not intended to indicate any dominant or recessive relationship. The fact that the lethal has no effect when present doubly in *tectorum* and that it exhibits its characteristic effect when present singly in the hybrid precludes any decision as to which, if either, is dominant.

Any discussion of the peculiar mode of behavior of this lethal factor must be purely speculative. Its lethal effect appears to be the result of failure of development at a stage when differentiation is the characteristic process. The ineffectiveness of the lethal in *tectorum* might indicate that this failure to develop normally involves some relationship between the gene complexes of the parental species. It has been shown (HOLLINGSHEAD 1928) that a plant will develop in a normal manner with a haploid complex of *capillaris* and it is likely that the same would apply to *tectorum*. It was suggested by BABCOCK (1924) that the mode of development determined by the haploid complex of the one species was incompatible with that determined by the haploid complex of the other. This hypothesis, however, does not explain why there is no apparent incompatibility between the mode of development determined by the *tectorum* complex containing the lethal allelomorph and that determined by the *capillaris* complex, nor between the modes of development of the *tectorum* complexes containing respectively the lethal and its allelomorph. If such an explanation was

the correct one it would mean that the mode of development determined by the *tectorum* complex containing the lethal allelomorph is compatible with either that determined by the *capillaris* complex or by the *tectorum* complex containing the lethal but that the last two are incompatible with each other.

The ineffectiveness of the lethal in *tectorum* might be explained by assuming an inhibitor. The lethal effect in the hybrid could be accounted for by assuming two genes in *capillaris* corresponding to those in *tectorum*, one recessive to the lethal and one dominant to the inhibitor. While without experimental foundation such an explanation is not inconceivable in view of the fact that GOULDEN (1925) and THOMPSON (1928) have found factors inhibiting the effect of dwarfing genes in wheat, and corresponding genes in two species of Drosophila have been established (MORGAN, BRIDGES and STURTEVANT 1925). Such an explanation would involve the assumption of the same or similar corresponding genes in the two species (*setosa* and *taraxacifolia*) which give hybrids unaffected by the lethal.

SUMMARY

Interspecific hybrids of *Crepis capillaris* and *C. tectorum* previously investigated developed only to the cotyledon stage. In renewed attempts to secure viable hybrids using different strains of the parental species one hybrid population was obtained of which half matured normally and half were inviable. Investigation showed that the *tectorum* parent had been heterozygous for a lethal factor which halted the development of the hybrid to which it was transmitted at the cotyledon stage. Progeny of the *tectorum* plant were all normal but on crossing the various plants to *capillaris* it was evident that the progeny were segregating for the lethal in a 1 : 2 : 1 ratio. Plants homozygous for the lethal allelomorph (*L L*) gave only viable hybrids, those homozygous for the lethal (*l l*) gave only inviable hybrids, and those heterozygous for it (*L l*) gave hybrids half viable, half inviable. Progenies from plants of each genotype were grown and their constitution tested by crossing with *capillaris*. All the progeny of *L L* proved to be *L L* giving viable hybrids only. Progeny of *L l* segregated in a 1 *L L* : 2 *L l* : 1 *l l* ratio giving hybrid populations of three kinds: (1) all viable, (2) half viable, half inviable, (3) all inviable. All the progeny of *l l* were *l l* giving only inviable hybrids.

The viability of the hybrids was independent of the *capillaris* parent used and there was no differential germination with respect to the lethal.

The lethal and its allelomorph have been found in *tectorum* strains from two widely separated localities.

There is evidence that the same lethal is effective in hybrids between *tectorum* and two other Crepis species, but hybrids with a third and fourth are unaffected by it.

The lethal may have played a rôle in maintaining ancestral stocks during the early differentiation of the species.

No experimental evidence is available to explain the ineffectiveness of the lethal in the parental species and its effect on an interspecific hybrid but an explanation is proposed which involves an inhibitor of the lethal and corresponding genes in the parental species.

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PROOF THAT THE ENTIRE CHROMOSOME IS NOT ELIMINATED IN THE PRODUCTION OF SOMATIC VARIATIONS BY X-RAYS IN DROSOPHILA

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I have elsewhere pointed out (1928, 1929a, 1929b) that two effects are produced by X-rays in the somatic cells of *Drosophila melanogaster*. One effect is the production of gene mutations, the other, that of breakages in the chromosomes. The effects are both revealed as variant areas in the soma, and such areas are usually identical in appearance whether they are due to breaks or to gene mutations. The chief difference is, in the case of sex-linked characters, that the variant areas in males are the result of gene mutations alone, while those appearing in the females (heterozygous for a sex-linked factor) may result from either cause.

This point may be made clear by citing a single experiment. If normal red-eyed females are crossed to white-eyed mutant males, and their larval offspring X-rayed, a certain proportion of the F_1 flies will show white variant areas or patches on their eyes. Each variant area is composed of a definite number of white ommatidia, and will be large or small, depending on whether early or late stages are treated. This means that if a mutation or breakage is induced in an early somatic cell, the descendant cells will produce an area showing the modification. The number of such areas in the males is found to be only about one-tenth as great as those found among the heterozygous females. Since, in such a cross, the male cell carries the gene for the dominant color factor in the single χ-chromosome present, and since the loss of this factor by breakage of this chromosome would result probably in the death of the cell, a white area on the eye of the male is interpreted as being the result of a gene mutation. The female cell has two χ-chromosomes, and, in the heterozygous condition, one of these carries the dominant color factor and the other carries the recessive factor. If a break occurs in the red-bearing chromosome, and the broken off piece with the dominant factor is lost, a white variant area will be produced, because of the presence of the gene for white in the homologous chromosome. If gene mutations occur in male cells, they must occur with equal frequency in female cells. The excess of white areas found among

the heterozygous females must therefore be due to breakages, or to the elimination of the entire red-bearing χ-chromosome.

An extensive series of experiments has been performed the results of which support the interpretation just given, but up to the present it has not been possible to demonstrate experimentally and conclusively that only a piece and not the whole chromosome is eliminated. Before presenting what may be regarded as a crucial experiment, we shall give such other evidence as seems to support our conclusion—that only a part of the chromosome is lost.

THE EVIDENCE FROM TRANSLOCATIONS

Much evidence, both genetic and cytological, has been obtained by MULLER and PAINTER (1929a, 1929b) showing that when a piece of the chromosome has been broken off by irradiating germ cells it may become attached to another non-homologous chromosome. Such cases are called "translocations." MULLER and PAINTER have been able to detect the attached piece, as well as the remainder of the chromosome from which the piece was broken off. They have worked out a number of such cases and have found that any one of the large chromosomes can be broken by X-rays, resulting in the formation of various kinds of translocations. In somatic cells some broken off pieces could become lost, either in the cytoplasm, or in the case of a translocation or duplication occurring in chromosome halves at a stage when all chromosomes are split, by passing into a sister cell. When a break occurs in a sex chromosome and a piece is lost in the cytoplasm the left-hand piece must be the one that is lost, for it is known that the mantle fiber is attached to the end conventionally represented on the right in the chromosome map. The term break, as used in this paper, carries with it the implication that the broken-off piece is lost, either in the cytoplasms, or through a fractional translocation.

THE EVIDENCE FROM THE EOSIN TEST

In connection with a series of experiments on the mutant eosin the results obtained were of such a nature that some of the variant areas must have followed breaks in the χ-chromosome (PATTERSON 1929a). One line of evidence (admittedly of an unsatisfactory and inconclusive nature) found in this series appeared in the variant areas which follow the loss of the gene for white in the somatic cells of females heterozygous for eosin and white. The eye color of the heterozygous eosin female is a light-eosin, like that of the eosin male, and not dark-eosin as in the homozygous female. The variant areas found among the heterozygous females were of

two kinds, white and dark-eosin. The white areas are due to the loss of the eosin factor or to gene mutations, and the dark-eosin areas were interpreted as probably being due to the loss of the gene for white by breakage. If the entire white-bearing chromosome had been eliminated, then the resultant area would have been light-eosin, and its detection would have been impossible on a light-eosin background. We have assumed that the loss of the gene for white through breakage upsets the previous genic balance between the two χ-chromosomes, with the result that the remaining portion of the broken chromosome so interacts with the factor for eosin in the other chromosome as to produce a dark-eosin area (PATTERSON 1929a). On the other hand, it is also possible that the dark-eosin areas arose through non-disjunction of the halves of an eosin-bearing chromosome, with the resultant formation of a cell containing three χ's, two bearing eosin and one bearing white; the sister cells would then have contained only one χ, bearing white.

More decisive evidence was found on examination of the results of raying larvae from pure eosin stock. Here it was found that no more light-eosin areas occurred on the eyes of the females than were to be expected as a result of gene mutations. If the cases of chromatin loss had involved the entire chromosome here, we should have observed a relatively great number of light-eosin areas due to this cause, since the eosin eye color produced by a single χ (as in the male) is known to be lighter than that produced by two χ's (as in the homozygous female). The absence of any light-eosin areas, except those due to gene mutations, thus shows that the cases of chromatin loss must have involved only a section of the chromosome, that is, breakage, and that a cell with a broken chromosome, lacking the gene for eosin, and one whole chromosome containing eosin, produces as dark a color as a cell with two whole eosin-bearing chromosomes.

THE EVIDENCE FROM THE WHITE-LOZENGE TEST

One experiment which further demonstrated that at least some of the white areas were the result of breaks in the χ-chromosome was the white-lozenge test. In this experiment normal red eyed females were crossed to white-lozenge males. Lozenge is also a sex-linked eye character, and its gene is located in the same chromosome as the gene for white. The locus of the gene for lozenge is at locus 27.7 on the chromosome map; it is therefore located 26.2 units to the right of the locus for white (which is at point 1.5). A total of 724 heterozygous females were obtained from treated larvae. It was calculated that among all the white areas found, thirty white-non-lozenge and 129 white-lozenge areas were due to breakage or to the

elimination of the entire red-bearing chromosome. But the thirty cases of white non-lozenge areas must have been produced by a break in the chromosome at some point between the locus for the dominant color factor and the locus for the normal allelomorph of lozenge; otherwise the lozenge character would have appeared in these thirty areas. In the case of the 129 white-lozenge areas, evidently the break occurred to the right of point 27.7, or else the entire red-bearing chromosome was lost.

While the above mentioned tests furnished conclusive evidence that breakage was the cause of many of the variant areas appearing in the females, yet there still remained the possibility that others of them might be due to the loss of the entire chromosome, and realizing this we made several further attempts to settle the question. One of these may be mentioned. Any crucial test would require the use of a sex-linked eye character with its gene location at or near the right-hand end of the chromosome. It just so happened that we had a new mutant eye color, called "carnation" (produced by raying larvae), the gene for which is at point 65.5. This is close to but not at the extreme right-hand end. In making tests with this character it was soon found that "carnation areas" could not be detected with certainty, because the carnation color is almost as deep as that of the normal red. At this point Doctor MULLER suggested that I try one of his "duplication" stocks. The results from this test are given in the next section.

THE THETA TEST

The duplication stock in question is known as "χ-duplication I," but we shall call it *Theta*. It has attached to the extreme right-hand end of of the sex-chromosomes, in fact, beyond the mantle fiber—as PAINTER'S cytological preparations have demonstrated—a small piece carrying the factor for gray body color, derived from the left-hand end of another sex chromosome. The stock is maintained by crossing heterozygous *Theta* females to yellow red-eyed males. This is done because the homozygous *Theta* females are very infertile. The composition of the sex-chromosomes of the *Theta* females is shown in diagram "A" of the accompanying figure. One of the χ-chromosomes (χ') has, in addition to the factors for yellow body color and red eyes, both of which are located at the left-hand end, the dominant allelomorph for body color, "gray," located at the extreme right-hand end in the small attached fragment. The other χ-chromosome (χ) is identical to its homologue, except that it does not have the extra fragment. Such a female has red eyes and a gray body, and when mated to, yellow red male will give *Theta* females, like their mother, yellow red males, yellow red females, and some *Theta* males.

In making the test for breakage, *Theta* females were crossed to yellow white singed males (instead of to yellow red males), and the early larval stages X-rayed. The composition of the sex-chromosomes of the *Theta* females found in the F_1 generation is shown in diagram B. The *Theta* females have one chromosome with the duplication (χ'), as before, but the other sex-chromosome carries yellow, white and singed (χ). The second class of females, or yellow red, has as before yellow and red in one χ-chromosome, and the yellow-white singed combination in the other χ-chromosome (figure 1, diagram E).

The test was made by treating larval stages and then examining the F_1 females for the different kinds of variant areas. The tabulated results are shown in table 1. There were 188 *Theta* females and 260 yellow females. There were fifteen gray-singed areas, fourteen white areas, four yellow

TABLE 1

Variant areas in females treated in larval stages, from a cross between Theta females and yellow white singed males.

CULTURE	AGE	DOSE	THETA FEMALES				YELLOW FEMALES		
			NUMBER	GRAY-SIN AREAS	WHITE AREAS	YELLOW AREAS	NUMBER	YEL-SIN. AREAS	WHITE AREAS
A	7–19	D–3	7	1	0	0	13	0	0
B	8–20	D–3	13	2	0	1	12	1	0
C	9–21	D–3	3	0	0	0	12	0	0
D	12–24	D–3	19	2	1	0	16	0	2
E	18–30	D–3	5	0	2	0	21	1	2
F	19–31	D–4	48	2	2	1	48	1	9
G	24–36	D–3	72	7	8	2	98	1	8
H	24–36	D–4	18	0	1	0	29	1	1
I	31–43	D–3	3	1	0	0	11	0	1
Totals			188	15	14	4	260	5	23
Controls			176	0	0	0	262	0	0

(non-singed) areas, and no yellow-singed areas found among the *Theta* females, and five yellow-singed areas and twenty-three white areas found

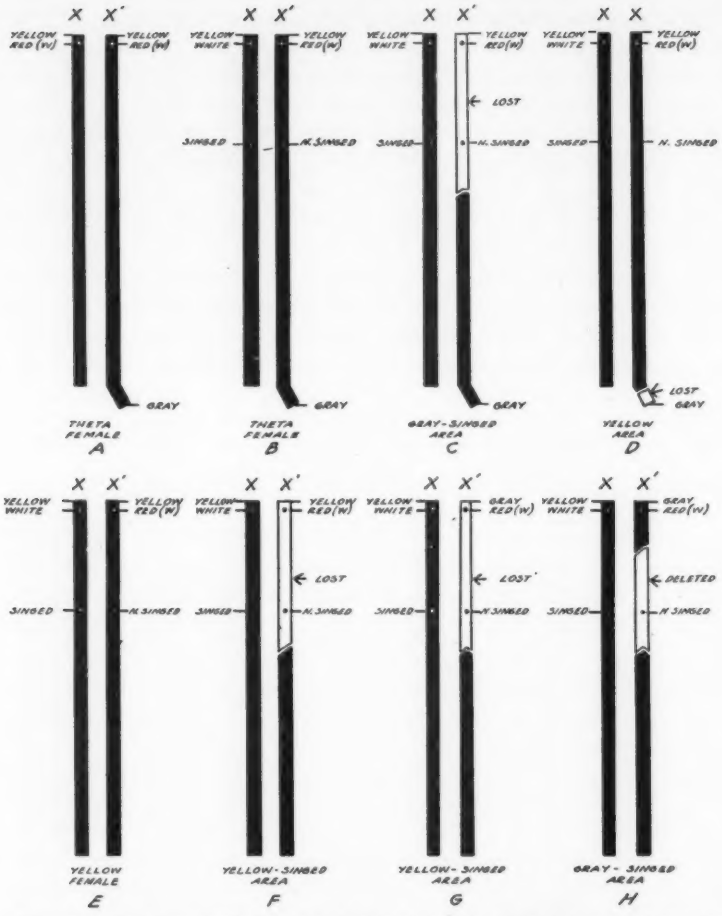

FIGURE 1.—Diagrams showing the composition of the pair of homologous sex chromosomes in cells of females used in the experiments. X is the chromosome from the father and X' is the sex chromosome from the mother. A, heterozygous *Theta* female of stock flies. B, heterozygous *Theta* female used in experiment. C, Gray-singed area of *Theta* female. D, yellow area of *Theta* female. E, heterozygous yellow female. F, yellow-singed area of yellow female. G, yellow-singed area of heterozygous wild-type female. H, gray-singed area of heterozygous wild-type female. The latter is a case of deletion. The attached fragment, as represented in diagrams A to D, is not drawn to the same scale as the rest of the chromosome. The main part of the chromosome is drawn as shown in the genetic maps, whereas the fragment is shown as proportionately larger, although genetically it is very small (see figure 8, MULLER and PAINTER 1929a).

among the yellow females. The white areas on the eyes in both types of females are similar to those previously described, and will not be considered further in this paper. The yellow, yellow-singed, and gray-singed areas are found on the body of the fly, and include bristles and hairs. It is these areas that are of interest in this connection.

We may now point out the meaning of these particular types of areas. A gray-singed area on a *Theta* female means that the χ-chromosome carrying the attached fragment was broken at some point to the right of the locus of the gene for the normal allelomorph for singed bristles (figure 1, diagram C). The fact that the singed areas are gray shows that the extra attached fragment is present. If they had been yellow, instead of gray, it would have indicated that the entire chromosome had been lost. All of the fifteen singed areas found among the *Theta* females were gray, showing that in no case was the whole chromosome lost. This test furnishes indisputable evidence in favor of our conclusion, namely, that the variant areas produced in somatic tissues by X-rays are in the main the results of breaks in the chromosomes.

The four cases of yellow areas found on the *Theta* females are of interest because they indicate that the small attached fragment, carrying the dominant color factor for gray body, has been broken off and lost (figure 1, diagram D). The reason why the number of these areas is so few, as compared to the number of gray-singed areas, is due to the fact that there is but a short distance in which a break can take place in the fragment. We should therefore expect to find a much greater number of singed areas because of the greater distance over which a break can take place in the main body of the chromosome and still produce such areas.

In addition to the twenty-three white areas found among the yellow females, there were also five yellow-singed areas. The method by which such areas would be produced by breakage is illustrated in diagram F.

Another interesting question that arose in connection with the *Theta* test is whether the left-hand end of the chromosome is always eliminated, rather than a section from the middle of the chromosome. This point may also be determined. The test was made by crossing normal red eyed females to yellow white singed males, and treating the larvae. The results of this test are shown in table 2. The 275 F_1 females gave sixteen white areas, 7 yellow-singed areas, one gray-singed area, and two yellow areas. We are concerned with the two types of singed areas. Seven out of the eight found were yellow. This means that the left-hand end had been lost (figure 1, diagram G). The origin of the single gray-singed area may be explained in the following manner. It may have resulted from a loss of a section of

that part of the X-chromosome which carries the factor for non-singed (figure 1, diagram H). Such cases are called "deletions," and it is supposed that the two remaining portions of the chromosome fused together at the time the loss occurred, as found by MULLER and PAINTER in the case of germ cell rayings. It is also possible that the gray-bearing fragment became attached to some other chromosome. In that case a break between

TABLE 2

Variant areas in females treated as larvae, from a cross between normal females and yellow singed white males.

CULTURE	AGE	DOSE	FEMALES	WHITE AREAS	YEL.-SINGED AREAS	GRAY-SINGED AREAS	YELLOW AREAS
A	8–20	D–3	15	1	0	0	0
B	20–32	D–3	93	1	4	0	0
C	24–36	D–3	51	3	1	0	1
D	24–36	D–4	101	9	2	1	1
E	31–43	D–4	15	2	0	0	0
Totals			275	16	7	1	2
Controls			226	0	0	0	0

non-singed and the fiber-bearing end of the chromosome need not have occurred, if this entire section became lost, but, as the other evidence in this paper indicates, loss of the fiber-bearing end rarely if ever occurs.

Throughout this paper we have not discussed the possibility that breaks may take place in the sex chromosome which carries the genes for the sex-linked recessive characters (the chromosome marked X in all of the diagrams). Such breaks must and do occur, but in the experiments given in this paper their effects could not be detected because of the presence of the dominant factors in the homologous chromosome. Experimental proof that both chromosomes break with equal frequency was obtained and presented in connection with our work on the mutant eosin, to which reference was made above.

We have not heretofore referred to the control flies. These numbers are listed in the two tables. As these data show, no variant area was found among any of the control flies.

CONCLUSION

The evidence presented in this paper supports the conclusion that, in addition to the production of gene mutation, X-rays cause breaks in the chromosome in the somatic cells of Drosophila. In the case of the somatic cells of females, heterozygous for a sex-linked character, the effect is revealed as a variant area which shows the recessive character only. This is due to the fact that that portion of the χ-chromosome which carries the gene for the dominant factor (or factors if more than one is involved) has been broken off and has become lost. This allows the recessive factor in the homologous chromosome to reveal itself in the soma as a variant area, composed of the descendant cells of the original affected cell. One may not be justified in saying that variant areas are always thus produced, for it may still be argued that the entire chromosome is occasionally lost. However, the "*Theta* test" described above demonstrated that in all cases there observed (19) only a part of the chromosome was lost.

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GAMETOPHYTIC INHERITANCE IN SPHAEROCARPOS. IV. FURTHER STUDIES OF TUFTEDNESS AND POLYCLADY

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In previous papers dealing with the genetics of *Sphaerocarpos Donnellii* Aust., the possibility has been pointed out of an analysis of inheritance by means of the distribution of genetic tendencies among the persistently adherent spores formed from a single spore mother cell. In work thus far reported the possibility has been realized only in a limited way, largely because of the difficulty of securing germination of all the spores of a tetrad. With reference to 2 vegetative gametophytic characters, as well as sex, a considerable amount of material is now available which, although far from satisfactory in various respects, seems to justify an attempt to use this method of analysis on a larger scale than hitherto. Certain definite conclusions are possible from the results here presented. In considerable measure, however, they are but suggestive of future possibilities.

The work reported in this paper has been carried on with the assistance, at different times, of MR. JAMES A. LOUNSBURY, DOCTOR GEORGE O. COOPER, Miss BEATRICE I. NEVINS, DOCTOR OPHELIA C. WESLEY, and Miss INEVA F. REILLY. This assistance was made possible by grants from the research fund of the UNIVERSITY OF WISCONSIN.

THE TUFTED CHARACTER

In an earlier paper (ALLEN 1924) the occurrence and characteristics of "tufted" clones were discussed in detail. Apart from some aberrancies in form of thallus lobes and in habit of growth, the outstanding peculiarity of a tufted gametophyte is a tendency for its archegonial or antheridial involucres to depart, often to an extreme degree, from the typical form. The character is a variable one; some or many of the branches in a tufted clone are indistinguishable from those of a typical clone; other branches bear both typical and variant involucres, and on still others all the involucres are markedly atypical.

Tufted clones were isolated on a number of occasions from material received from Sanford, Florida. Races of this character are apparently not uncommon, at least in that locality.

The previous study has shown that tuftedness is inherited vegetatively; even a strictly typical branch of a tufted clone gives rise sooner or later to tufted branches. The tufted character is likewise inherited through gametes and spores; that is, tufted and nontufted clones are genotypically different. It has also appeared that between tufted clones there are genetic differ-

ences, indicated by the appearance in different clones of very different proportions of typical and tufted branches. Cases were cited in the previous paper illustrating the fact that, while the same clone may at different times include varying proportions of tufted branches, nevertheless some clones consistently over a term of years present a very high proportion, others a median, and still others a very low, proportion of such branches.

If several factors exist for tuftedness, it might be expected that these factors, present singly, would produce different phenotypic results. Repeated study of numerous tufted clones with this possibility in mind has, however, disclosed no diagnostic differences, save in the degree to which the tufted tendency is expressed.

The method has been described by which the proportion of tuftedness of a given clone is calculated. At each observation, an estimate is made of the proportion of the area of the soil in a three-inch pot covered by the clone; and of the proportion of tufted branches (that is, of branches bearing preponderantly atypical involucres). From the figures so obtained, the proportion, expressed as a percentage, is determined of the number of tufted branches observed to the total number of branches present in the clone at the times of all observations, successive observations having been at least three months apart. Percentages, when given in the present paper, are stated as the nearest whole numbers; in case the calculated percentage is less than 0.5, it is given as "slight" ("sl."). Since the calculation is based upon estimates, and since the actual proportion of tuftedness characterizing a particular clone varies from time to time, probably in response to environmental changes, it is evident that the percentages given must be considered only as rough approximations. With all due allowances, however, they afford some measure of the genetic differences which seem clearly to exist.

The percentages will be noted to range from "slight" to considerably over 90 percent. In no case has a tufted clone which lived for any considerable time failed to produce at least an occasional typical branch. At the opposite extreme are clones which, during a life of several years, have been observed to produce only one or a very few tufted branches. At this extreme, obviously, the distinction between a genetically tufted and a genetically nontufted clone is uncertain, since the former may not have been observed to bear tufted branches. Some tufted clones have, therefore, doubtless been classed as nontufted; and the occurrence of such an error is the more likely the shorter the period of life of such a clone. The opposite error is much less likely but not impossible, because the distinction may not always be certain as between a rare tufted branch and one with

several atypical involucres which still falls within the limits of variability of a nontufted race. This difficulty is illustrated by the classification at first adopted of "possibly atypical" clones. Later work (ALLEN 1926) has shown that some such clones, phenotypically of doubtful character, are genotypically nontufted. The whole picture thus afforded is that of a continuous series (or one of an incalculable number of terms) connecting an extreme of remarkably uniform ("typical") character with the opposite extreme of very high but never quite uniform deviation from the typical condition.

Another source of possible error is the division of a sporeling into 2 before its isolation from its tetrad sibs. The majority of cases of this nature noted were among the offspring of spore tetrads sown in the autumn of 1923. In consequence of my absence from Madison during part of the following winter and the spring, some of these sporelings were separated only after they had attained a relatively large size. The possibility of a previous division was thus of course increased. Even under most favorable circumstances, however, an error of this nature is not excluded.

Such an error is detected if it results in the appearance of more than 2 female, or more than 2 male, clones coming from a single tetrad. In each such case the error was corrected in the tabulation of results, as noted in the discussion of the respective matings. The occurrence of 3 clones of 1 sex might in some instances suggest an actual deviation from the ordinary sex ratio (of 2 females and 2 males from each tetrad). However, none of the cases of this nature noted in the present work seems to be susceptible of such an interpretation; sometimes because the total number of apparently separate sporelings from a tetrad was more than 4; more often because circumstances noted at the time of isolation of certain sporelings cast doubt upon their separate origin. It seems safe therefore to assume that, so far as my work to date is concerned, every tetrad, had all its spores germinated, would have given rise to 2 female and 2 male clones.

A similar error might remain undiscovered if, for example, only one of the genetically female spores of a tetrad were to germinate, the resultant sporeling then becoming divided. Such errors, if, as is possible, they have at times occurred, have not seriously affected the results discussed in the present paper for the reason that, as shown in the tabulations, in the large majority of cases the 2 female, or the 2 male, clones arising from a tetrad are genetically similar. In a minority of cases, as will appear, the distribution of the polycladous—nonpolycladous character pair is distinct from that of the sexual characters, the result being the production of four genetically different gametophytes from a tetrad. Apparently a similar inde-

pendent segregation may occur involving sex and tuftedness. An error of the sort in question might tend to reduce the apparent proportional occurrence of such an intra-tetrad distribution.

In the first paper of this series (ALLEN 1924) the results were compared of matings of nontufted×nontufted, and of tufted×nontufted. In the work here reported, the study has been extended to the offspring of nontufted×tufted, and of tufted×tufted. The tufted males used as parents were descendants of the tufted females of previous matings. A comparison has been made also between the genetic behavior of little-tufted and much-tufted clones.

Since gametophytic characters only are being considered, the maternal and paternal clones used in the respective matings (the p_1 generation) will usually be referred to as "parents," and their gametophytic descendants of the f_1 generation as "offspring." Strictly speaking, of course, the p_1 gametophytes are the *grandparents* of the f_1 gametophytes, the immediate offspring of the former being F_1 sporophytes.

Nontufted ♀ ♀ ×*Much-tufted* ♂ ♂

The female clones used in the experiments of this series were all strictly nontufted so far as numerous observations over considerable periods have disclosed. The male clones were selected as showing a large proportion of tuftedness.

The date of fertilization given in each case is that at which portions of the female and male clones in question, after being placed in the same pot, were first flooded with sterilized water. Since the flooding was usually repeated several times at intervals of one to several days, the actual date of fertilization may have been, in some instances, later than that given.

Mating 19. 21.215 (nontufted)×21.16 (75 percent tufted)

Fertilized May 12, 1922. Mature sporophytes dried July 7. Twenty spore tetrads from each of 2 sporophytes sown separately October 20 and 24. Sporelings transplanted February 12–March 24, 1923.

The mother in this mating was an offspring of mating 1 (nontufted× nontufted; ALLEN 1924). At the time of 3 observations during its first year's growth it showed a rather large proportion of irregularities; numerous observations during the succeeding 6 years, however, have shown it to be typical. This was one of the clones which were first classed as "possibly atypical," but later results (ALLEN 1926) showed several such clones to be genetically similar to others classed as "typical."

The father was an offspring of mating 14 (tufted×polycladous; ALLEN

1925). The structures of this clone were at times, as previously reported, so aberrant as to suggest that it might be genetically polycladous as well as tufted. But, since none of its progeny have shown the polycladous character (in the present mating and in mating 20, reported below), it is clearly to be classed as tufted. The percentage of tuftedness to be ascribed to it has been more than usually doubtful; roughly it is placed at 75 percent.

The results of the present mating as well as those of mating 26 have been provisionally reported (ALLEN 1924a). Such differences as appear in the distribution of the offspring of these 2 families between table 2 of the earlier paper and tables 1 and 3 of the present report result in part from the fact that a few clones earlier classed as typical have later shown themselves to be tufted, and in part from the omission (in the present paper) of a few clones whose determination seemed unsatisfactory in view of their early death. No clone is here classed as nontufted which was not so determined as a result of at least two observations some months apart.

In 1 instance in this family, referred to previously (ALLEN 1924a, p. 228), 2 groups of 2 plants each appeared in 1 pot. In 1 group were 1 nontufted female and 1 plant of undetermined sex; in the other group, 1 nontufted female and 1 female of undetermined character. These were omitted from the previous tabulation; they are included in the present one as representing 2 tetrads, each yielding (so far as determinations were possible) 1 nontufted female. In another pot, 2 distinct groups appeared, each consisting of 2 females and 2 males. It seemed clear that 2 separate spore tetrads were here involved, and the results are so treated. One tetrad, also noted in the earlier paper, apparently gave rise to 1 nontufted female, 1 tufted male, 1 nontufted male, and 1 male of undetermined character. At the time of transplanting, it was noted that the last-named male and the male classed as nontufted were possibly parts of the same plant. That is here considered to have been the case.

One female nontufted clone (23.110) has shown a large proportion of involucral appendages as well as some minor irregularities in the forms of the involucres. While appendages occur rarely on the involucres in many clones that are otherwise typical, a few female clones and at least 1 male clone had previously appeared in which such appendages were characteristically numerous. This character has been briefly discussed (ALLEN 1924, pp. 576, 577). The difficulty of studying its inheritance is increased by the fact that involucral appendages are frequent in the tufted clones; in a clone with a tufted ancestry like the one under consideration, it is possible, therefore, that the appendages are an expression of the tufted tendency. But since, during 6 years, no tufted branch has been seen in

clone 23.110, it is provisionally classed as an "appendiculate" mutant.

The offspring in this family from the tetrads of two sporophytes are grouped together in table 1. No significant differences have been noted between the offspring of distinct sporophytes derived from the same mating.

Mating 20. 21.116 (nontufted)×21.16 (75 percent tufted)

Fertilized May 12, 1922. Sporophytes dried July 12. Ten tetrads from one sporophyte sown November 3. Sporelings transplanted March 29 and April 9, 1923.

The mother in this, like that in the previous mating, was an offspring of mating 1. Numerous observations have determined it without question as nontufted. The father was the same as in mating 19.

Comparatively few spores resulting from this mating germinated (table 1), and some of the f_1 clones died while young. It is probable that the apparently small proportion of tufted offspring is to some extent misleading.

Mating 21. 21.224 (nontufted)×20.60 (89 percent tufted)

Fertilized February 10, 1923. Sporophytes dried May 1. Forty-eight tetrads from one sporophyte sown September 18. Sporelings transplanted December 22, 1923–January 27, 1924.

The mother in this case was an offspring of mating 1. It has shown a uniformly typical character. The father was an offspring of mating 8 (tufted, separable×nontufted; ALLEN 1924). The high percentage of tuftedness assigned it is an average of 19 observations showing respectively from two-thirds to more than nine-tenths tufted branches.

In 5 instances in this family the germinations from a tetrad apparently resulted in more than the 2 expected females. This is 1 of the families with reference to which the records show that some groups of the sporelings were so far advanced in growth when they were transplanted as to make their separation difficult. For reasons already outlined these cases were treated, for purposes of classification, as follows:

(a) Four females, all nontufted; considered as 2 nontufted females. (b) Similar to a. (c) 2 tufted females, 1 nontufted female, 1 tufted male; considered as 1 tufted female, 1 nontufted female, 1 tufted male. (d) 1 tufted, 2 nontufted females; considered as 1 tufted, 1 nontufted. (e) 3 tufted females. At the time of transplanting, the third plant was noted as possibly a fragment of the second; it was so considered in the classification.

With these emendations, the results of this mating are as shown in table 1. The nontufted "appendiculate" female clone (23.5114) occurring in this family is similar to clone 23.110 descended from mating 19.

Mating 22. 21.116 (nontufted) × 20.60 (89 percent tufted)

Fertilized February 24, 1923. Sporophytes dried April 26. Sixty tetrads from one sporophyte sown September 20. Sporelings transplanted January 27–February 1, 1924.

TABLE 1

Results of matings of nontufted × much-tufted; distribution of offspring according to spore tetrads from which they arose (M. = mating; t. = tufted; nt. = nontufted).

f_1 ♀	f_1 ♂	M.19	M.20	M.21	M.22	TOTALS	
(a) 2 nt.	1 t.	2	..	1	1	4	
(b) 2 nt.	....	1	2	2	1	6	
(c) 1 nt.	1 t.	1	..	1	..	2	
(d) 1 nt.	....	3	3	4	4	14	
(e)	2 t.	..	..	1	..	1	
(f)	1 t.	1	..	2	..	3	30
(g) 2 t.	2 nt.	3	..	..	..	3	
(h) 2 t.	1 nt.	2	..	1	..	3	
(i) 2 t.	....	7	..	4	1	12	
(j) 1 t.	2 nt.	1	..	..	..	1	
(k) 1 t.	1 nt.	1	..	1	..	2	
(l) 1 t.	....	1	1	3	2	7	
(m)	2 nt.	..	..	1	..	1	
(n)	1 nt.	..	..	1	2	3	32
(o) 2 nt.	2 nt.	2	..	1	..	3	
(p) 2 nt.	1 nt.	2	..	..	..	2	
(q) 1 nt.	2 nt.	..	1	..	1	2	
(r) 1 nt.	1 nt.	..	1	..	2§	3	
(s) 1 nt., 1 t.	2 nt.	2	..	..	..	2	
(t) 1 nt., 1 t.	1 nt.	2	..	..	..	2	
(u) 1 nt., 1 t.	....	2	..	3†	2	7	
(v) 1 nt.	1 nt., 1 t.	1	..	..	..	1	
(w)	1 nt., 1 t.	..	1	..	..	1	23
(x) 1 nt., 1 t.	1 t.	1*	..	1	1	3	
(y) 2 t.	2 t.	1	..	..	..	1	
(z) 2 t.	1 t.	..	..	1	..	1	5
Totals		36	9	28	17		90

Total no. ♀ nontufted clones, 66; ♀ tufted, 64; ♂ nontufted, 41; ♂ tufted, 19. Character not determined: ♀, 3; ♂, 30; sex undetermined, 4. Totals, all sporelings: ♀, 133; ♂, 90; sex unknown, 4; grand total, 227.

* Nontufted ♀ is "appendiculate" (probably a mutant).

† One nontufted ♀ is appendiculate.

§ One nontufted ♂ is a dwarf mutant.

The mother was the same as in mating 20; the father, the same as in mating 21. The results are shown in table 1.

In one instance in this family the offspring from a single spore tetrad consisted of 1 female and 3 males, all nontufted. This group is classed as 1 nontufted female, 2 nontufted males.

One case of an undoubted mutant occurs in this family. It is a male clone (23.6002), substantially resembling a typical male except that all parts, including the involucres, are much smaller. No other similar plant has been observed. In 5 matings with as many different female clones this dwarf male has failed to produce sporophytic offspring.

Like previous studies on Sphaerocarpos, the present one gives no indication of a departure from the rule that, if all spores of a tetrad germinate, 2 give rise to female, and 2 to male, gametophytes. The apparent exceptions, as already noted, are explainable, most of them obviously so, as due to an early separation of a single plant into 2.

As pointed out in previous papers, if segregation of chromosomes or of chromosome parts is limited to the heterotypic division in the spore mother cells (and if the distinction between tuftedness and nontuftedness behaves as though due to a single factorial difference) the tetrads in matings like those here considered would be expected to be of 2 classes: those yielding on germination 2 nontufted females, 2 tufted males; and those yielding 2 tufted females, 2 nontufted males. Of the tetrads whose offspring are reported in table 1, those in series a to f inclusive belong or may belong to the former class (allowing for the failure of many of the spores to germinate); and those in series g to n may belong to the latter class. That the tetrads of these two classes are substantially equal (30 : 32) is perhaps significant; for equality would be expected if there is no linkage between tuftedness (or its reverse) and sex.

The 28 tetrads in series o to z inclusive (table 1) do not seem to fall in either of the "expected" classes. With reference to a large proportion of these apparently exceptional tetrads, however, the possibility of an error in classification is to be considered, the error, namely, resulting from the failure of a genetically tufted clone to produce tufted branches. Such an error is especially likely in the case of a clone which died before it had reached a considerable size, and on which but a limited number of observations were made. Among the clones represented in the series in question were many, especially among the males, which died young. So far as concerns series o to w, it is clear that corrections might be made by changing individual clones from the nontufted to the tufted category so that each of

the 23 tetrads included in these series would be referred to one of the two classes represented respectively by series a to f and series g to n. This would reduce the number of apparently exceptional tetrads from 28 to the 5 included in series x, y, and z. That such a correction should be made for many of the male clones is indicated by the discrepancy in total numbers between male nontufted (41) and male tufted clones (19), as compared with the near-equality in number between female nontufted and female tufted clones (66 : 64).

On the other hand, it is not clear that all the tetrads in series o to w should thus be removed from the "exceptional" category. In a few of the apparently "exceptional" cases, the clones, or certain of the clones, classed as tufted have been in existence so long, and have been examined so often, that it seems improbable that, if genetically tufted, they should not have betrayed the fact. While certainty can not be attained, the possibility is not to be dismissed that 3 or even 4 nontufted clones may develop from a spore tetrad in such a family as one of those here considered.

With reference to the possibility of the occurrence of more than 2 tufted clones among the offspring of a single tetrad, illustrated by series y and z, there is less doubt. As already pointed out, while a tufted clone may for a long time produce no tufted branches and may therefore be classed as nontufted, a genetically nontufted clone never produces tufted branches. The 2 cases falling within these series, therefore, in spite of the smallness of the number, indicate that now and then among the offspring of a mating of tufted by nontufted may appear a tetrad 3 or all 4 of whose spores carry the tufted potentiality.

Still another possibility is suggested by series t to x, table 1. This is the development of 2 tufted and 2 nontufted clones from a single tetrad, 1 male and 1 female being tufted and 1 male and 1 female nontufted. A precisely similar distribution of the polycladous and nonpolycladous characters independently of sex, resulting in the production of a small proportion of "four-type" tetrads, has been clearly established (ALLEN 1924a, 1926a), and such distributions will be discussed on a later page. With reference to tuftedness the evidence is less conclusive, because of the ever-present possibility of the error of determining a tufted clone as nontufted. However, in view of the very confident determinations of the characters of some of the clone tetrads concerned, as well as of the certain recognition of a corresponding distribution in the case of the polycladous character, it is concluded provisionally that in a minority of cases a spore tetrad may give rise to 1 nontufted and 1 tufted female, 1 nontufted and 1 tufted male.

Little-tufted ♀♀ × *Little-tufted* ♂♂

Mating 23. 20.29 (8 percent tufted) × 20.103 (9 percent tufted)

Fertilized February 12, 1923. Sporophytes dried June 26. Spores (separate) from 1 sporophyte sown September 23. Sporelings transplanted December 5, 1923–January 26, 1924.

Both parents used in this mating were among the offspring of mating 8 (ALLEN 1924). The mother in mating 8 was the clone (R27E) whose sporophytic offspring were the first observed to produce separate spores (ALLEN 1925a). Since the spore-separation tendency is transmitted through all the female descendants of clone R27E, the sporophytes resulting from mating 23 likewise produced separate spores. It is therefore impossible to classify the progeny by tetrads. The gametophytic offspring of this mating were:

Females: Nontufted, 6; tufted, 12; character undetermined, 1.

Males: Nontufted, 6; tufted, 1; character undetermined, 12.

Total females, 19; total males, 19; sex and vegetative character undetermined, 6; grand total, 44.

There was a large proportion of early deaths of clones in this family, especially among the males; this fact accounts for the number whose character was in whole or in part undetermined.

Mating 24. 20.310 (6 percent tufted) × 20.312 (12 percent tufted)

Fertilized March 22, 1923. Sporophytes dried May 8. Forty-six tetrads from one sporophyte sown September 17. Sporelings transplanted December 22, 1923–January 26, 1924.

Forty-eight tetrads from a second sporophyte sown October 24, 1924. Sporelings transplanted February 14–March 27, 1925.

Both parents in this mating were among the offspring of mating 3 (tufted by nontufted; ALLEN 1924). Both have shown consistently a low proportion of tuftedness, except that on 3 occasions small sub-clones of the male parent consisted only of typical or approximately typical branches.

Five tetrads showed apparently aberrant conditions. These were:

(a) A tetrad giving 2 nontufted females, 3 tufted males. When the sporelings were first transplanted, it was noted that 2 of the male plants were closely associated and possibly originally parts of 1 plant. These 2 males are here considered as a single clone.

(b) A tetrad giving 3 tufted females, respectively 1, 2, and 3 percent tufted. The latter 2 are considered as a single clone.

(c) A tetrad giving 3 tufted females, 1 nontufted male. Of the 3 females,

the 2 most closely similar as to proportion of tuftedness (respectively 38 and 39 percent) are treated as a single clone. The tetrad is thus classed as as having yielded 2 tufted females, 1 nontufted male.

(d) A tetrad giving 3 nontufted females, 1 tufted male. The 3 females are considered as representing 2 clones.

(e) A tetrad giving 3 nontufted females; these are treated as 2 clones.

With the emendations noted, the results of mating 24 are shown in table 2, the offspring of spore tetrads from the 2 sporophytes being classed together.

Mating 25. 20.310 (6 percent tufted)×20.122 (2 percent tufted)

Fertilized March 22, 1923. Sporophytes dried May 11. Fifty-four tetrads from 1 sporophyte sown October 16, 1924. Sporelings transplanted January 7–March 23, 1925.

The mother was the same as in mating 24. The father was an offspring of mating 8 (tufted, separable×nontufted; ALLEN 1924).

A large proportion of clones in this family (19 ♀ ♀, 26 ♂ ♂, 4 of undetermined sex) died before their vegetative characters were satisfactorily determined. The number of tetrads that could be classified as to the charac-

TABLE 2

Results of matings of little-tufted×little-tufted; distribution of offspring according to spore tetrads.

f_1					
♀	♂	M.24	M.25	TOTALS	
2 nt.	2 t.	2	..	2	
2 nt.	1 t.	3	..	3	
2 nt.	....	6	2	8	
1 nt.	1 t.	3	..	3	
1 nt.	....	12	5	17	
....	1 t.	2	1	3	36
2 t.	2 nt.	3	1	4	
2 t.	1 nt.	3	..	3	
2 t.	....	6	1	7	
1 t.	1 nt.	2	..	2	
1 t.	....	12	4	16	
....	1 nt.	3	..	3	35
Totals		57	14		71

Totals (matings 23, 24, 25): ♀ nontufted clones, 52; ♀ tufted, 58; ♂ nontufted, 22; ♂ tufted, 14. Character not determined: ♀, 21; ♂, 50; sex undetermined, 10. Totals, all offspring: ♀, 131; ♂, 86; sex unknown, 10; grand total, 227.

ter of their offspring is therefore, as appears in table 2, comparatively small.

One clone gave 3 males of undetermined character; they are treated as 2 clones. Another tetrad gave 3 females and 1 male, all of undetermined character; they are treated as 2 females, 1 male.

In families 24 and 25, as shown in table 2 (mating 23 being unanalyzable in terms of tetrads), the character of the offspring is exactly what would be expected from a mating between a tufted and a nontufted parent, on the assumption that the parental difference was due to a single factor. The 2 groups of tetrads expected in such a case—namely, those which *may* be composed respectively of 2 nontufted females, 2 tufted males, and of 2 tufted females, 2 nontufted males—are substantially equal. Table 2, showing the offspring of 2 tufted parents, is thus exactly comparable with the upper 2 divisions of table 1, showing the offspring of matings of nontufted with tufted parents, but lacks entirely the really or apparently aberrant types of intra-tetrad distribution shown in the lower part of table 1. In other words, the results of the mating of 2 tufted parents agree more closely with the expectation, on a simple Mendelian basis, as to the results of the mating of 1 tufted and 1 nontufted parent than do the results of the latter type of mating itself. This unexpected condition might be explained by the assumption that in the matings now in question 1 of the parents had been erroneously classified, having been really nontufted; but the history of both parents in each case negatives such a possibility. Another conceivable explanation would be that the offspring classed as nontufted were really tufted. As previously pointed out, an error of this nature is bound to occur at times in the determination of the character of genetically slightly tufted clones; but the present results are too uniform, and the history of many of the offspring clones has been followed too long, to leave any plausibility in such an assumption. So far as possible, the apparently nontufted offspring were kept in culture much longer than most of those that were obviously tufted, to increase the chance that they might produce tufted branches. A few of the nontufted female clones, indeed, are still living.

Little-tufted ♀ × *Much-tufted* ♂

Mating 26. 21.14 (1 percent tufted) × 20.384 (82 percent tufted)

Fertilized May 12, 1922. Sporophytes dried July 7. Twenty tetrads from 1 sporophyte sown separately December 2. Sporelings transplanted March 29–May 18, 1923. The distribution of the offspring is shown in table 3.

Tetrads from a second sporophyte sown broadcast December 2, 1922. Sporelings transplanted April 5 and 13, 1923. Clones arising from these sporelings were classed as follows:

Females: Nontufted, 12; tufted, 10; character undetermined, 3. Males: nontufted, 8; tufted, 1; character undetermined, 8.

Tetrads from a third sporophyte sown broadcast December 2, 1922. On April 13, 1923, numerous sporelings arising from this sowing were discarded. They included apparently nontufted males, and tufted and nontufted females.

Both parents in this mating were among the offspring of mating 14 (tufted×polycladous; ALLEN 1925). The mother on July 1, 1921 showed two branches that were classed as tufted. Several later examinations failed to disclose any tufted branches, and it was concluded that the earlier observation had been erroneous. For this reason the clone was treated as "typical" in a previous paper (ALLEN 1924a). On July 27, 1927, tufted branches were again present. The clone must therefore now be considered as tufted.

TABLE 3

Results of mating of little-tufted×much-tufted; distribution of offspring according to spore tetrads.

f_1		M.26	
♀	♂		
2 nt.	2 t.	1	
2 nt.	....	3	
1 nt.	....	2	6
2 t.	1 nt.	1	
2 t.	....	5	
1 t.	1 nt.	1	
1 t.	....	1	8
1 nt., 1 t.	1 nt.	1	1
Total			15

Totals (offspring from spores of 1 sporophyte, shown above, plus spores of a second sporophyte sown broadcast): ♀ nontufted, 23; ♀ tufted, 25; ♂ nontufted, 11; ♂ tufted, 3. Character not determined: ♀, 4; ♂, 15. Totals, all sporelings: ♀, 52; ♂, 29; grand total, 81.

One tetrad of the present family, as noted previously, apparently gave rise to 3 female clones, all tufted. Estimates of the proportions of tuftedness displayed by these clones gave respectively 43, 57, and 59 percent. The two latter, being so similar phenotypically, are assumed to have come from a single plant, and the tetrad is listed as having produced 2 tufted clones.

It will be seen from table 3 that the results of this mating of little-tufted ×much-tufted, so far as can be judged from the relatively small numbers, do not differ materially in character from those shown in previous tables for matings of nontufted×much-tufted or of little-tufted×little-tufted.

The 1 "exceptional" tetrad shown in table 3 illustrates the difficulties of classification of clones that are phenotypically near the border line between tuftedness and nontuftedness. One female clone (23.245) from this tetrad was kept in culture for nearly 3 years. On one occasion it showed a few undoubtedly tufted branches; at all other times when it was examined it appeared nontufted. It is classed as 1 percent tufted. The second female clone from the same tetrad (23.246) is still living, being now more than 6 years of age. It has never shown a tufted branch, although on 1 occasion a few "doubtful" branches (showing some marked irregularities), and on another occasion 1 doubtful branch, were observed. It is classed as nontufted. The 1 male clone of this group (23.247) died after but 2 observations of its character had been made; on both occasions it displayed no tufted branches, and it is therefore classed as nontufted. Obviously its determination is not entirely satisfactory.

Much-tufted ♀♀ ×*Much-tufted* ♂♂

Mating 35. 20.331 (82 percent tufted)×20.320 (64 percent tufted)

Fertilized February 24, 1923. Sporophytes dried May 5. Thirty-six tetrads from one sporophyte sown September 14. Sporelings transplanted December 21, 1923–January 26, 1924.

Both parents were among the offspring of mating 3 (tufted×nontufted; ALLEN 1924). Both produced throughout their lives large proportions of tufted branches.

The offspring of this mating were transplanted when somewhat advanced in growth. In consequence, it appeared from the characters of the respective clones that in a number of instances a plant had been divided before being transplanted. In 3 instances, 3 female clones appeared from a single tetrad instead of the expected 2; in one case there were 4 female clones instead of 2; and in 3 instances 3 male clones appeared instead of 2. Corrections were made in these cases, as previously described. The occurrence of so many errors in isolation of clones makes it not unlikely that now and then the 2 female or the 2 male clones referred to a tetrad may represent really but 1 clone. In this family, however, the results are so uniform that such errors can not affect the conclusions, except as they might somewhat modify the calculated average percentages of tuftedness (table 5).

The results of this mating are given in table 4.

Mating 36. 20.331 (82 percent tufted)×20.111 (74 percent tufted)

Fertilized February 24, 1923. Sporophytes dried May 3. Twenty tetrads from 1 sporophyte sown September 14. Sporelings transplanted December 5, 1923–January 25, 1924.

The mother was the same as in mating 35. The father was an offspring of mating 8 (tufted, separable×nontufted; ALLEN 1924).

The results are shown in table 4.

Mating 37. 20.313 (55 percent tufted)×20.107 (93 percent tufted)

Fertilized February 24, 1923. Sporophytes dried May 8. Forty-five tetrads from 1 sporophyte sown October 17, 1924. Sporelings transplanted February 13–March 24, 1925.

The mother in this mating, like that in mating 35, was an offspring of mating 3. The father, as in mating 36, was an offspring of mating 8.

Germinations in this case, as table 4 shows, were relatively few, and most of the sporelings died while young.

Mating 38. 20.173 (77 percent tufted)×20.252 (71 percent tufted)

Fertilized March 8, 1923. Sporophytes dried May 16. Forty-eight tetrads from 1 sporophyte sown October 26, 1924. Sporelings transplanted March 17–28, 1925.

Both parents were among the offspring of mating 14 (tufted×polycladous; ALLEN 1925).

Only a few germinations were obtained. Of the 2 male clones listed in table 4, 4 observations of 1 clone, and 3 of the other, showed them nontufted.

Mating 39. 20.173 (77 percent tufted)×20.384 (82 percent tufted)

Fertilized March 8, 1923. Sporophytes dried May 16. Forty-eight tetrads from 1 sporophyte sown October 26, 1924. Sporelings transplanted February 14–March 28, 1925.

Both parents in this mating were among the offspring of mating 14 (tufted×polycladous). The mother was the same as in mating 38; the father the same as in mating 26.

One tetrad resulting from mating 39 yielded apparently 3 male and 1 female clones. Of the males, 2 died before their character was determined; the third, on 4 observations, appeared nontufted. These are treated as 2 males, 1 nontufted and 1 of undetermined character. The female clone from this tetrad was polycladous. The possibility of contamination seems to be excluded, since at its first transplanting from the pot in which the

tetrad was sown this female plant was noted as "apparently polycladous" —a determination confirmed by 2 later observations when the clone was large enough to make the diagnosis certain. It must therefore be considered a mutant; possibly the fact may be significant that the mutation was a return to the character of one of its gametophytic great-great-grandparents. The results, including the 3 tetrad sibs in question, are shown in table 4.

TABLE 4

Results of matings of much-tufted×much-tufted.

f_1 (BY TETRADS)	NUMBER OF TETRADS					
	M.35	M.36	M.37	M.38	M.39	TOTALS
2♀, 2♂	5	1	..	..	..	6
2♀, 1♂	6	6	..	..	3	15
2♀ ...	3	1	..	..	1	5
1♀, 2♂	6	2	..	1	1	10
1♀, 2♂, 1?	1	..	..	..	..	1
1♀, 1♂	2	3	1	..	..	6
1♀ ...	3	4	4	..	8	19
... 2♂	1	..	..	..	1	2
... 1♂	1	1	1	..	1	4
... 1♂, 1?	..	..	1	..	..	1
... ... 3?	..	..	1	..	..	1
... ... 1?	..	..	6	5	1	12
Totals	28	18	14	6	16	82

Character of f_1 Offspring.

	M.35	M.36	M.37	M.38	M.39	TOTALS
♀ tufted	40	25	2	1	16	84
♀ nontufted	..	..	..	..	1*	1
♀ undetermined character	..	..	3	..	..	3
♂ tufted	29	9	..	..	4	42
♂ nontufted	..	..	..	2	1	3
♂ undetermined character	6	7	3	..	3	19
Sex and character undetermined	1	..	10	5	1	17
Totals	76	41	18	8	26	169

* Polycladous mutant.

Table 4 shows that the results of matings of much-tufted×much-tufted are materially different from those of matings of the types previously considered. Of the f_1 female clones whose character was determined with some confidence, only 1 of 85 was classed as nontufted; and that 1 was a mutant in which tuftedness may have been masked by polyclady. Of f_1 males similarly determined, only 3 in 45 were classed as nontufted. The possible failure of a slightly tufted clone to produce tufted branches over a considerable period renders it not unlikely that any or all of these 3 clones may have been genetically tufted. Apart, therefore, from rare exceptions which may involve mutation, matings between much-tufted clones (of the character of those used in the present work) give entirely tufted offspring. This result would naturally be expected of a mating between any 2 tufted clones, at least on the assumption that they bear a common factor for tuftedness; but it is, in fact, very different from the consequences that followed matings of little-tufted×little-tufted (table 2) or of little-tufted× much-tufted (table 3). It follows that clones displaying different degrees of tuftedness differ in their genetic make-up, as evidenced by the character of their offspring.

Genotypic differences between parents of different degrees of tuftedness are indicated also by the percentages of tuftedness manifested by their offspring, as shown in table 5 for each of the families thus far considered.

In considering this table, comparison may first be made of the range and percentage of tuftedness characterizing the tufted offspring alone of each group of families. Inspection of the table shows notable differences in these respects, particularly between the offspring of much-tufted×much-tufted and the offspring of matings of other types. Further light is thrown on these differences by the average tuftedness of the offspring in each group of families. The averages are as follows, computed for the tufted offspring only:

For females: offspring of nontufted×much-tufted, 11 percent; little-tufted×little-tufted, 15 percent; little-tufted×much-tufted, 35 percent; much-tufted×much-tufted, 45 percent.

For males: offspring of nontufted×much-tufted, 30 percent; little-tufted×little-tufted, 45 percent; little-tufted×much-tufted (3 clones only), 35 percent; much-tufted×much-tufted, 49 percent.

For all tufted offspring: nontufted×much-tufted, 15 percent; little-tufted×little-tufted, 21 percent; little-tufted×much-tufted, 35 percent; much-tufted×much-tufted, 47 percent.

The percentages given for female clones are more dependable than those given for males; partly because the number of females is larger, partly be-

TABLE 5

Percentages of tuftedness in offspring of matings involving tuftedness.

		NUMBER TUFTED CLONES	RANGE OF TUFTEDNESS (PERCENT)	AVERAGE (PERCENT)	NUMBER NONTUFTED CLONES	TOTAL NUMBER (CHARACTERS DETERMINED)	AVERAGE ALL CLASSIFIED CLONES (PERCENT)
Nontufted × Much-tufted							
M.19	♀	36	Sl.–63	14	26	62	8
	♂	8	5–43	26	24	32	6
M.20	♀	1	....	1	9	10	Sl.
	♂	1	.....	41	4	5	8
M.21	♀	20	Sl.–46	9	17	37	5
	♂	8	1–98	39	7	15	21
M.22	♀	7	Sl.–10	3	14	21	1
	♂	2	4–9	6	6	8	1
Little-tufted × Little-tufted							
M.23	♀	12	Sl.–53	23	6	18	15
	♂	1	.....	38	6	7	5
M.24	♀	38	Sl.–39	11	37	75	6
	♂	12	15–90	44	14	26	20
M.25	♀	8	2–50	19	9	17	9
	♂	1	.....	66	2	3	22
Little-tufted × Much-tufted							
M.26	♀	25	1–95	35	23	48	18
	♂	3	6–48	35	11	14	8
Much-tufted × Much-tufted							
M.35	♀	40	12–96	57	0	40	57
	♂	29	9–74	41	0	29	41
M.36	♀	25	Sl.–69	27	0	25	27
	♂	9	34–86	65	0	9	65
M.37	♀	2	20–75	48	0	2	48
	♂	0	.....	..	0	0	..
M.38	♀	1	.....	17	0	1	17
	♂	0	.....	..	2	2	0
M.39	♀	16	15–77	47	1*	17	44
	♂	4	58–87	71	1	5	57

* Polycladous mutant.

cause a small proportion of tuftedness is less likely to be noted in a male clone.

The figures, making all allowance for the unsatisfactoriness of the absolute values given, render it abundantly evident that there are genetic differences between clones which differ phenotypically in their proportion of tuftedness. The argument would obviously be strengthened if comparison were made between the percentages of tuftedness averaged for *all* offspring, tufted and nontufted, in the respective groups (see the last column of table 5). Such a comparison is, of course, valid only as between the last 3 groups of families, both parents in each of which were tufted.

Likewise suggestive of genetic complexity are the differences in proportion of tuftedness between members of the same family, shown in the column of table 5 headed "range of tuftedness." These differences are emphasized by a comparison of the records of individual clones within a family; while some show marked discrepancies between the results of observations at different times, many clones have displayed consistently over a term of years a uniform tendency toward, respectively, a high, medium, or low degree of tuftedness.

While the averages show a general correspondence in degree of tuftedness between parents and offspring, despite great differences between individual offspring, the correspondence may be less close when comparison is made within single families. Thus, in families 23 and 25, the tufted female offspring were on the average more tufted than either parent, and the single male clone in each family was similarly highly tufted. In these families, as well as in family 24, the maximum tuftedness among either female or male offspring was several times the tuftedness of either parent. Even when the average is calculated to include nontufted as well as tufted offspring (last column, table 5), the degree of tuftedness is higher than that of either parent in the females of family 23, in the males of family 24, and in both females and males of family 25. Among the offspring of matings 35, 36, and 39, in which both parents were much-tufted, the maximum proportion among the offspring of one or the other sex exceeds the proportion of either parent, although the differences here concerned may fall within the limits of possible error in the determination of degrees of tuftedness.

It has been suggested (ALLEN 1924, pp. 565, 566) that a factorial explanation for tuftedness requires the assumption that more than 1 factor is or may be concerned in the production of this character. The original basis for the suggestion was the observation that different clones persistently manifest different degrees of tuftedness. Clones selected as vary-

ing markedly in degree of tuftedness have now been shown to differ in their genetic behavior.

The occasional production from a mating between a nontufted female and a much-tufted male of a tetrad giving rise to 3 or 4 tufted gametophytes (table 1) indicates the presence in the male parent of more than 1 factor determining tuftedness. The results of matings of much-tufted × much-tufted (table 4) point likewise to the action of 2 or more distinct and cumulative factors, present in this instance in each parent. That the factors borne respectively by the 2 parents in each such mating are not identical is indicated by the differences among the offspring with respect to degree of tuftedness. Were the parents genetically alike, a greater uniformity among the offspring would be expected. If the factors borne by the mother were entirely different from those borne by the father, some proportion of the offspring would be expected to be nontufted. It is true that a very few of the offspring (in 2 families) were so classified, but reasons have been given for suspecting that their classification was erroneous. Hence it is plausible to assume that in each of these matings the parents had 1 or more factors for tuftedness in common, each possessing in addition 1 or more factors that were lacking in the other parent.

However, the genetic character of a majority of the tetrads resulting from a mating of nontufted × much-tufted (first 2 divisions of table 1) could be explained by the assumption of a *single* differentiating factor. If 2 or more such factors are present in each male parent, a close linkage between these factors must be postulated in order to explain the occurrence of so large a proportion of tetrads each yielding 2 nontufted and 2 tufted clones.

An alternative line of explanation, and one which better fits the results of certain other matings, would assume the presence of at least 1 factor of another type. In the matings summarized in table 1, it may be supposed that each much-tufted father bears 2 or more factors for tuftedness ($T_1, T_2, \ldots$) which may or may not be linked, and in addition a factor R which does not tend in itself to produce tuftedness but is necessary to the appearance of that character. It must also be assumed that R is closely linked with one of the factors of the former group (say T_1). In such a case the majority of the tetrads would give rise each to two nontufted and two tufted clones; and crossing over between T_1 and R would account for an occasional tetrad producing nontufted clones only. The apparently rare production of 3 or 4 tufted clones from a single tetrad might depend upon an additional factor R', present in certain much-tufted males and closely

linked with T_1 and R, or upon an occasional mutation of r, derived from the nontufted mother, to R.

The results of matings of much-tufted×much-tufted (table 4) offer no difficulty to conceptions of the nature of those just developed. A crossing over between R and T_1 in the one parent, or between similar factors in the other parent, might result in the appearance of an occasional nontufted clone; otherwise all the f_1 clones would be tufted.

To account on a similar basis for the consequences of matings of little-tufted×little-tufted (table 2), or of little-tufted×much-tufted (table 3), further assumptions are required. In the matings of the former type it may be supposed, as before, that 1 parent possesses the factor-complex RT_1, R being closely linked with T_1 and with other similar factors, if present, but that the other parent has the complex $R'T_n$, R' not being linked with T_n or with any other factor affecting tuftedness. An F_1 sporophyte, then, would have the formula $R\,r\,R'r'\,T_1t_1\,T_nt_n$. (Capital and lower case symbols here, of course, do not imply dominance and recessiveness, since characters of the haploid generation are in question.) Leaving out of consideration the consequences of a rare crossing over between R and T, as well as the possible occasional occurrence of a four-type tetrad, tetrads of the following constitutions would be formed in equal numbers: $2RT_1R'T_n$, $2\,rt_1r't_n$; $2RT_1r'T_n$, $2rt_1R't_n$; $2RT_1R't_n$, $2rt_1r'T_n$; $2RT_1r't_n$, $2rt_1R'T_n$. The first, second, and third of these combinations would give rise to 2 tufted clones of 1 sex, 2 nontufted of the opposite sex; the fourth combination, to 4 tufted clones, 2 of either sex. Three-fourths of the tetrads, therefore, would belong to the 2 classes appearing in table 2; one-fourth to a class not there shown to occur. It may be argued that, since the number of tetrads shown in the table is comparatively small, and since in so large a proportion of cases only 1 or 2 spores of a tetrad germinated, giving rise to 1 or 2 tufted clones, it is not impossible that some fraction of the tetrads resulting from these matings were actually genetically all tufted. This possibility, that of the occurrence of crossing over, and that of the appearance of four-type tetrads, require testing by means of much larger families.

The hypothesis could be further developed by assuming additional factors necessary, in addition to R', for the appearance of tuftedness; with appropriate assumptions as to linkage, the expected proportions of tetrads producing all tufted clones could thus be reduced indefinitely.

An explanation thus developed, while perhaps formally satisfactory, requires many assumptions that are as yet unsupported. On the other hand, certain results of matings involving the "semisterile" and "cupulate"

characters have suggested the occurrence in Sphaerocarpos of a method of inheritance which may possibly play a rôle in the phenomena here under discussion.

Both semisterility and cupulateness have appeared in male gametophytes only. The former, distinguishing several clones that probably had a common origin, was described by WOLFSON (1925). The latter character has appeared once in a mutant (from mating 31, described later in the present paper). Both characters are constant and are inherited indefinitely through vegetative multiplication; semisterile clones have been in continuous culture since 1921; the cupulate clone, since 1924. None of the offspring of matings of semisterile or of cupulate males have displayed the paternal character. For example, a mating of a semisterile male with a tufted female gives rise to tufted and nontufted gametophytes of either sex, but to none that show the semisterile character. The possible explanation that spores carrying the semisterile tendency are nonviable is negatived by the fact that in some instances germination has been secured of 3 or of 4 spores from a single tetrad. Equally conclusive evidence is not yet available from matings involving the cupulate character.

More or less closely analogous occurrences of matrocliny in angiosperms have been explained by cytoplasmic influence; either the material basis of inheritance (for example, of plastid characters) is thought to reside in the cytoplasm of the egg, or the maternal cytoplasm is considered to exert an inhibiting influence upon the expression of factors contributed by the male gamete. It is possible that an explanation of this general nature may apply to the unexpected results of matings involving semisterility and cupulateness. Or, quite differently, the X-chromosome contributed by the egg may be found to play some preponderant rôle in inheritance proportional to its bulk.

Returning to the results of matings between little-tufted and little-tufted (table 2), and of that between little-tufted and much-tufted (table 3), it is clear that their explanation would be much simplified if it could be supposed that a factor or a factor-complex received from 1 parent may be inactivated or its expression inhibited in consequence of gametic union. This supposition might leave the results of all the matings involving tuftedness explainable in terms of multiple factors determinative for various intensities of that character. The supposed inactivation of factors coming from 1 parent could not, however, apply to all the factors furnished by that parent, nor even to all the factors affecting tuftedness; for, in matings involving a high degree of tuftedness on both sides (table 4), if all such factors in either parent were to become functionless, some con-

siderable proportion of the offspring would be expected to be nontufted; and this is clearly not the case. Likewise, in certain matings involving polyclady reported later in the present paper, in which 1 parent was genetically tufted, there is no evidence of a suppression in the offspring of the tufted tendency, from whichever parent that tendency was inherited.

THE POLYCLADOUS CHARACTER

Polycladous plants, as already described (WOLFSON 1925, ALLEN 1925), differ from typical gametophytes in frequency of lobing and branching; in the forms of lobes and of involucres; and in the presence of numerous dorsal lobes and cilia. The males bear fewer antheridia than typical males, considerable portions of a culture often lacking antheridia entirely. The females bear only extremely rare archegonia and in numerous attempts at mating have proved entirely sterile. Polyclady, differently from tuftedness, is constant in expression. A polycladous clone, male or female, produces only polycladous branches, although rarely an involucre appears of approximately typical form. All my polycladous plants are descended from two male clones which probably had a common origin.

Previous work has shown that polyclady behaves in matings as though the difference between it and the typical condition depended upon a single factor. With this conclusion the present results are in agreement.

The apparent simplicity of the method of inheritance of this character suits it particularly for the study of the intra-tetrad distribution of the genetic factors involved. In table 6 all the material available to date for such an analysis is brought together. The figures repeated in this table from previous papers are those from matings 12–15 (ALLEN 1925) and 28–32 (ALLEN 1926a). Two corrections have been made in the report of mating 32, as noted below in the discussion of that mating.

From matings involving both tuftedness and polyclady (ALLEN 1925), the expected class of offspring showing both these characters could not be recognized. It was concluded provisionally that "the aberrancies due to the inheritance of tuftedness are masked by the greater and uniformly present aberrancies of polyclady." This conclusion is confirmed by the results of matings 27 and 31–34 (below); the three polycladous males shown by these results to be also genetically tufted were among the progeny of mating 14 (nonpolycladous, tufted×polycladous). In table 6, only the results bearing upon the inheritance of polyclady (or its absence) are included.

Mating 40 involves also the character known as "appendiculate." The distinction between appendiculate and non-appendiculate clones is difficult

and requires the study of more, and more prolific, matings than have yet been obtained. Its discussion is therefore deferred to a later report.

In the cases of 3 sowings [from matings 27, 32, and 34, all nonpolycladous, nontufted×polycladous (tufted)] made on October 21, 22, and 23, 1923, it was evident from the numbers of sporelings appearing that 2 or more sets of tetrads had been sown in some or all of the pots. Since uncertainty was thus introduced as to intra-tetrad distribution, the results of these sowings, though summarized in table 8, are omitted from the classification in table 6.

Nonpolycladous ♀♀ × *Polycladous* ♂♂

Mating 12. 19.24×R19δ1aB
Mating 13. 19.6×R19δ1aA

These matings were reported earlier (ALLEN 1925).

The tetrads from 1 sporophyte of mating 12, sown broadcast, gave only 1 sporeling, a nonpolycladous male.

Spore tetrads from 3 sporophytes resulting from mating 13 were sown separately. One spore from each of 4 tetrads germinated, 3 spores giving rise to nonpolycladous females, 1 to a polycladous male. Tetrads from 5 sporophytes, sown broadcast, gave more extensive results (table 1, ALLEN 1925). Since the tetrad relationships of the offspring of these spores could not be determined, they are not classified in table 6 but are summarized in table 8.

Mating 28. 20.337×21.148

Fertilized March 31, 1922. Sporophytes dried July 7. Twenty tetrads from 1 sporophyte sown November 8. Sporelings transplanted April 13–May 17, 1923.

Both parents were among the offspring of mating 13 (above). The mother in the present mating was one of the clones which, because of the presence at times of an unusual number of aberrant involucres, was classed provisionally as "possibly atypical." But the offspring of a mating of this clone with a typical male (mating 16, ALLEN 1926) showed it to be genetically indistinguishable from a typical clone.

Mating 29. 21.85×20.266

Fertilized May 12, 1922. Sporophytes dried July 7. Twenty tetrads from 1 sporophyte sown November 7. Sporelings transplanted April 12–May 3, 1923.

The mother was an offspring of a mating of typical parents (mating 1; ALLEN 1924). The father was an offspring of mating 13, noted above.

Mating 30. 21.153×20.266

Fertilized May 12, 1922. Sporophytes dried July 12. Twenty spore tetrads from 1 sporophyte sown January 12, 1923. Sporelings isolated May 18.

The mother in this mating was among the offspring of mating 13. It was one of those first classed as "possibly atypical," but its breeding behavior (mating 17; ALLEN 1926) showed it to be genetically indistinguishable from typical clones.

The father was the same as in mating 29.

Nonpolycladous, Appendiculate ♀ ×*Polycladous* ♂

Mating 40. PSC2Bδ1.8b×20.266

Fertilized February 10, 1926. Sporophytes dried May 21. Thirty-five tetrads from 1 sporophyte sown separately November 8. Sporelings isolated May 24–28, 1927.

The mother in this mating was an offspring of fertilization within a culture of male and female plants received from Miami, Florida, in 1916. This clone has been preserved because it has shown rather consistently an unusually large proportion of involucral appendages, as well as other minor irregularities in the form of its involucres. In the present paper, the question of the inheritance of the appendiculate character will not be considered.

The father in this mating was the same as in matings 29 and 30.

The classification of 1 male offspring (clone 27.210) is still somewhat uncertain. It resembles polycladous males in most respects, but produces an unusually large proportion of involucres approaching more or less closely the typical form. It has given sporophytic progeny in 1 mating with a typical female and a few spores have germinated. It is impossible to determine from the nature of the still young plants arising from these spores the genetic constitution of their male parent. This clone is classed in table 6 as polycladous. Since it was 1 of only 2 male progeny of mating 40, the other being nonpolycladous, the possibility is open that its character is a resultant of the presence of appendiculate and polycladous factors.

Nonpolycladous, Tufted ♀ ♀ ×*Polycladous, Nontufted* ♂ ♂

Mating 14. R35G1 (67 percent tufted)×R19δ1aA

Mating 15. R27E (53 percent tufted)×R19δ1aB

The details of these matings and the source of the parents were reported earlier (ALLEN 1925).

Thirty-two and 16 tetrads, derived respectively from 2 sporophytic

offspring of mating 14, were sown separately. The offspring arising from these spores are classified in table 6. Tetrads from a third sporophyte were sown broadcast. The results of their germination are included in table 8.

Since the mother in mating 15 carries the spore-separation tendency, the spores borne by its sporophytic progeny were not united in tetrads. Spores from 3 of these sporophytes were sown broadcast. Among the offspring of spores from 1 sporophyte were tufted and nontufted but no polycladous clones. Since a doubt is thus suggested as to the paternity of this sporophyte, its gametophytic offspring are not here recorded; those of the other 2 sporophytes are summarized in table 8.

Nonpolycladous, Nontufted ♀ ♀ ×*Polycladous* (*Tufted*) ♂ ♂

The mothers in this series of matings were all strictly typical. The fathers were among the progeny of mating 14 (above). Although they were not distinguishable phenotypically from other polycladous males, their nonpolycladous offspring include both nontufted and tufted clones. It is evident, therefore, that the polycladous fathers in the present matings were genetically tufted.

Mating 27. 21.45×21.13

Fertilized March 8, 1923. Sporophytes dried May 8. Forty-eight tetrads from 1 sporophyte sown October 23. Sporelings isolated January 24–March 27, 1924.

The mother was an offspring of mating 13; the father, of mating 14.

This is one of the families in which the numbers of sporelings appearing made it evident that in several instances 2 or more tetrads had been sown in a single pot. This family is therefore not included in table 6 but is summarized in table 8.

Mating 31. 20.205×20.254

Fertilized March 8, 1923. Sporophytes dried May 9. Sixty tetrads from 1 sporophyte sown September 27. Sporelings isolated January 17–February 5, 1924.

Both parental clones were among the progeny of mating 14.

One spore tetrad in this family apparently produced 3 nonpolycladous females; they are classed as 2 clones. One apparently produced 4 nonpolycladous males; they are classed as 2.

One male offspring (23.7094), classed in table 6 as nonpolycladous, is a new mutant form. The antheridia in this clone are about as abundant as in a typical clone, but are relatively small. The antheridial involucres,

while variable in form, are prevailingly expanded into cup- or saucer-like structures, at the center of each of which a partly exposed antheridium is attached. This mutant clone, referred to as "cupulate," has been mated with 6 different females. Sporophytes resulted from 2 of these matings; the nature of their gametophytic offspring has been noted on a previous page.

Mating 32. 20.205×20.255

Fertilized March 8, 1923. Sporophytes dried May 21. Forty-eight tetrads from 1 sporophyte sown October 21. Sporelings isolated January 22–February 5, 1924.

Twenty-four tetrads from a second sporophyte sown October 24, 1924. Sporelings isolated February 14–March 27, 1925.

Both parents were among the progeny of mating 14. The mother was the same as in mating 31.

Of the offspring of the spores sown in 1924, 7 appeared in one pot and 6 in another, although only 1 tetrad was supposed to have been sown in each. Since it was evident that each of these two pots at least had received more than 1 tetrad, all the results of the sowings of 1924 are omitted from table 6, but are included in table 8.

Among the offspring of the sowing of 1923 the following apparent discrepancies occurred. One tetrad yielded 2 female nonpolycladous, 1 female polycladous, 1 male polycladous; the 2 nonpolycladous females are considered as 1 clone. One tetrad yielded 3 nonpolycladous females, classed as 2 clones. One tetrad yielded 3 nonpolycladous females (classed as 2) and 1 polycladous male.

The classification of the offspring of this mating (sowing of 1923) as shown in table 6 differs from that given in a previous paper (ALLEN 1926a, table 1) in two respects. One tetrad previously classed as yielding 1 polycladous female, 1 nonpolycladous male is now credited with 1 nonpolycladous male only, because of the unsatisfactory determination of the (probable) female. One tetrad, yielding 1 nonpolycladous female, 1 polycladous female, 1 nonpolycladous male is included which was omitted from the previous tabulation.

Mating 33. 21.45×20.254

Fertilized March 8, 1923. Sporophytes dried May 11. Forty-eight tetrads from 1 sporophyte sown October 2, 1924. Sporelings isolated February 21–March 26, 1925.

The mother in this mating was the same as in mating 27. The father was the same as in mating 31.

One tetrad in this family yielded apparently 3 polycladous males (classed as 2) and 1 nonpolycladous female.

Mating 34. 21.45×20.255

Fertilized March 8, 1923. Sporophytes dried May 8. Forty-eight tetrads from 1 sporophyte sown October 22, 1924. Sporelings isolated January 7–March 26, 1925.

The mother was the same as in matings 27 and 33; the father, the same as in mating 32.

This family represents another instance in which more than 1 set of tetrads were evidently sown in the same pots. For this reason the results are not included in table 6, but are summarized in table 8.

TABLE 6

Results of matings of nonpolycladous×polycladous; distribution of offspring according to spore tetrads. (M. = mating; np. = nonpolycladous; p. = polycladous).

f_1 ♀	f_1 ♂	M. 12, 13	M. 14	M. 28	M. 29	M. 30	M. 31	M. 32	M. 33	M. 40	TOTALS	
2 np.	2 p.	..	..	..	..	..	4	2	..	..	6	
2 np.	1 p.	..	..	..	..	..	3	4	..	..	7	
2 np.	....	..	..	1	1	..	3	3	4	..	12	
1 np.	2 p.	..	..	..	1	..	2	1	3	..	7	
1 np.	1 p.	..	..	1	..	..	2	2	2	..	7	
1 np.	....	3	7	2	2	..	4	3	7	6	34	
....	2 p.	..	..	1	3	..	..	3	..	..	7	
....	1 p.	1	..	..	..	2	2	..	3	..	8	88
2 p.	2 np.	..	..	..	..	..	2	..	..	..	2	
2 p.	1 np.	..	..	..	..	..	..	3	..	..	3	
2 p.	....	..	1	..	..	..	..	1	..	1	3	
1 p.	2 np.	..	..	..	1	..	1	1	..	..	3	
1 p.	1 np.	..	..	1	3	..	4*	2	..	..	10	
1 p.	....	..	1	4	1	..	1	4	2	3	16	
....	2 np.	..	..	1	1	..	4	1	1	..	8	
....	1 np.	1	..	2	2	..	2	2	4	1	14	59
1 np., 1 p.	1 np., 1 p.	..	..	1	..	..	..	1	..	..	2	
1 np., 1 p.	1 p.	..	..	..	..	..	..	1	..	..	1	
1 np., 1 p.	....	..	..	..	..	..	1	..	..	..	1	
1 np.	1 np., 1 p	..	..	..	..	..	1	..	1	..	2	
1 np.	1 np.	..	..	..	..	..	1	..	1	..	2	
1 p.	1 np., 1 p.	..	..	..	..	..	..	2	..	..	2	
1 p.	1 p.	..	..	..	..	..	..	1	..	1†	2	12
Totals		5	9	14	15	2	37	37	28	12		159

* One "nonpolycladous" ♂ is a cupulate mutant.

† Polycladous ♂ of somewhat uncertain character.

One important difference is to be noted between the inheritance of polyclady and that of tuftedness. From matings of nontufted×tufted (table 1), the class of tetrads whose spores are genetically 2 nontufted female, 2 tufted male, and that class yielding 2 tufted females, 2 nontufted males seem to be approximately equal. On the other hand, from matings of nonpolycladous×polycladous (table 6), the corresponding classes of tetrads, one giving 2 nonpolycladous females, 2 polycladous males, the other 2 polycladous females, 2 nonpolycladous males, appear to be very unequal. In the latter case the results may be better judged if only those tetrads are considered whose genetic constitution is beyond question—on the assumption, supported by all the evidence now at hand, that of the spores of each tetrad 2 are genetically female and 2 male, 2 nonpolycladous and 2 polycladous. Accepting this assumption, the character of the spores of a tetrad is established if all 4, or only 3, germinated; or if 2 germinated, giving rise either to 2 female, 2 male, 2 nonpolycladous, or 2 polycladous clones. The tetrads represented in table 6, the germination of whose spores met one of these requirements, are of the 3 classes shown in table 7.

TABLE 7

Genetic types of spore tetrads (decisive cases only) among offspring of nonpolycladous×polycladous.

MATING	2np., ♂2p.	♀2p., ♂2np.	♀1np., 1p., ♂1np., 1p.	TOTALS
14	..	1	..	1
28	2	1	..	3
29	5	2	1	8
31	12	7	3	22
32	13	6	5	24
33	7	1	2	10
40	..	1	1	2
Totals	39	19	12	70
Percentages	56	27	17	100

The suggestions of table 6 are confirmed by table 7. It is shown that, among the tetrads produced by the sporophytic offspring of a mating of nonpolycladous×polycladous, those whose (genetically) female spores are nonpolycladous and whose male spores are polycladous are about twice as numerous as those in which the reverse relation holds between vegetative character and sex, and that the third class, whose four spores are all genetically different, are fewer than those of either of the two-type classes, these four-type tetrads constituting about one sixth of the total number. These proportions are not very different from those based upon more limited

numbers as given previously (ALLEN 1926a); at that time the numbers for the corresponding classes were respectively 32, 16, and 8. The chief difference resulting from the inclusion of additional results is that the proportion of four-type tetrads is raised from one seventh to about one sixth of the total.

Of the classes of tetrads in question, the first is that in which the character-pairs, femaleness-maleness and polyclady-nonpolyclady, are represented in each tetrad in the combinations in which they occurred in the gametophytic parents; the second class is that in which the parental characters appear in new combinations. That tetrads of the first class are about twice as numerous as those of the second implies a linkage of some nature between factors for sex and those determining polyclady or nonpolyclady, a severing of this linkage occurring during meiosis in about one third of the spore mother cells.

If sexual characters were not involved, the natural assumption would be that the two character-pairs concerned are represented by factors borne on the same pair of chromosomes, the breaking and recombination of their linkages being explainable by the familiar hypothesis of crossing over. But it has been shown (ALLEN 1919; LORBEER 1927) that the influences differential for sex in this species reside in the X- and Y-chromosomes. These chromosomes are so different in size as to make it difficult to imagine an intertwisting and an exchange of corresponding parts between them. It is at least equally possible that the partial linkage here demonstrated is one between different chromosomes, these chromosomes tending to segregate in such a manner in meiosis that the parental grouping (in the case of the X–Y and one other chromosome-pair) persists about twice as often as it is broken. A possible mechanism for such a persistence of parental chromosome groupings is suggested by the end-to-end connections of chromosomes observed in several plants and most fully studied by CLELAND (1923 and later papers) and others in Oenothera. That not all the chromosomes of Sphaerocarpos tend to retain their parental groupings during meiotic segregation is indicated by the inheritance of tuftedness or nontuftedness independently of sex.

The relation of the production of tetrads of the third (the four-type) class to occurrences during meiosis has already been discussed (ALLEN 1924a, 1926a). Segregation of chromosomes or of chromosome parts limited to the heterotypic division must produce a tetrad composed of spores of only 2 genetically different types. The occurrence of 4 types in a single tetrad implies, therefore, some qualitative segregation in the homoeotypic division. That segregation in the present case occurs ex-

clusively in the homoeotypic division is rendered unlikely, as already pointed out, by the relatively small proportional occurrence of four-type tetrads. It is negatived also by the extensive cytological evidence for the heterotypic segregation of chromosomes in plants, as well as by the demonstrated fact that in *Sphaerocarpos Donnellii* the X- and Y-chromosomes separate in the heterotypic division (ALLEN 1919; LORBEER 1927). It is concluded therefore that some form of segregation may occur in each division. A similar conclusion has been drawn by Miss NEWTON (1926) from her study of factors affecting syngamy in *Coprinus lagopus*. The segregation here in question may imply the separation of some chromosome pairs in the heterotypic, and of some pairs, regularly or occasionally, in the homoeotypic division (as described for some Metazoa; MCCLUNG 1914); or a regular segregation of chromosomes in the heterotypic division, preceded by a crossing over in the "four-strand" stage (BRIDGES 1916).

That four-type tetrads result also from matings involving tuftedness has been noted as probable in the discussion of the inheritance of that character.

Adopting the ratios found in table 7 between numbers of tetrads of the 3 possible classes, the proportion of *clones* of each type to be expected in an f_1 population derived from a mating of nonpolycladous × polycladous is 9 ♀ nonpolycladous: 5 ♀ polycladous: 5 ♂ nonpolycladous: 9 ♂ polycladous. Incidentally, exactly the same proportion of types will result if the ratio 4:2:1, based upon more limited results (ALLEN 1926a), holds as between the respective classes of tetrads.

Table 8 gives the classification of all the f_1 offspring yielded by matings of this nature, including both those which could and those which could not be classified according to tetrad relationships. Applying the proportions just calculated to the totals in table 8, it appears that the relative numbers among females are substantially as expected, the actual numbers being 259 nonpolycladous, 155 polycladous, whereas a 9:5 ratio would give 266 nonpolycladous, 148 polycladous. The males, however, do not fit the expectation; there are 173 nonpolycladous and 158 polycladous, instead of 118 and 213, as calculated from a 5:9 ratio. With respect to this discrepancy it is to be noted, first, that females are more vigorous than males and more likely to survive to a time when their character may be determined. They should, therefore, present a more accurate picture of the distribution of characters within a given population. Second, the preponderance of nonpolycladous males apparent in table 8 is due to the earlier matings (12–15); if only the later matings were considered, the balance would swing somewhat in favor of polycladous males (nonpoly-

cladous 106, polycladous 117). Third, because of the lesser viability of males, most if not all of the 11 polycladous clones whose sex was undetermined were probably males. Most of the 84 whose sex and vegetative character were both undetermined were also in all probability males; but no conclusion is justified as to whether the greater number of these were nonpolycladous or polycladous. Apparently some portion of the departure from the expected ratios must be explained by assuming that spores bearing the genetic bases for maleness and polyclady germinate in smaller propor-

TABLE 8

Results of matings of nonpolycladous × polycladous; total offspring.

MATING	♀ np.	♀ p.	♂ np.	♂ p.	p. (SEX?)	UNDETERMINED	TOTALS
12	..	..	1	..	..	..	1
13	46	28	28	21	3	11	137
14	17	9	9	7	..	11	53
15	34	30	29	13	3	28	137
27	19	14	14	12	..	4	63
28	6	6	6	4	..	..	22
29	5	5	9	8	..	2	29
30	..	..	..	2	..	..	2
31	31	11	22*	20	..	3	87
32	39	24	23	25	..	12	123
33	22	2	8	12	5	9	58
34	34	20	23	33	..	3	113
40	6	6	1	1†	..	1	15
Totals	259	155	173	158	11	84	840
Expected Proportions	9	5	5	9			

* Includes one cupulate mutant.

† Male of somewhat uncertain character.

tions than do those that are genetically male and nonpolycladous; or that polycladous males are less viable in their very early stages. The latter suggestion is somewhat weakened by the observed fact that in general, after they are well started in growth, polycladous are more resistant than nonpolycladous males to unfavorable conditions.

INTERRELATIONS OF TUFTEDNESS AND POLYCLADY

The fact that polyclady masks the appearance of tuftedness interferes with the determination of possible genetic relations between these two characters. However, from their distribution, so far as it may be observed, in tetrads resulting from matings in which each character was contributed by but 1 parent, certain conclusions are possible. For the purpose of such

a study, material is available from family 14 (nonpolycladous, tufted × polycladous, nontufted) and from families 31–33 [nonpolycladous, nontufted × polycladous (tufted)]. Those tetrads of these families which yielded at least 2 fully identifiable sporelings each are classified in table 9.

In this table the tetrads are arranged in 3 groups: (1) those certainly or possibly two-type tetrads giving rise to nonpolycladous females and polycladous males (series a-k); (2) certainly or possibly two-type tetrads, producing polycladous females and nonpolycladous males (series l-r); and (3) apparently four-type tetrads (series s-β). In harmony with the more extensive results based upon the distribution of polyclady alone, tetrads of the first group are in this instance twice as numerous as those of the second.

For purposes of further analysis, it may be assumed as before that the spores of each tetrad are, genetically, 2 nonpolycladous and 2 polycladous, 2 female and 2 male; and, in addition, 2 nontufted and 2 tufted. The latter assumption would fit the very large majority of cases, as shown by the results summarized in table 1, but leaves out of consideration the infrequent appearance, as shown in that table, of a tetrad of 3 or 4 genetically tufted spores (or possibly, also, of 3 or 4 nontufted spores). Omitting these rare occurrences, 4 classes of two-type tetrads and 8 classes of four-type tetrads may be expected to result from matings like those here in question.

Of the tetrads listed in table 9, some give definite results with reference to the 3 pairs of characters concerned; others do not. For example, in series a, b, and c of that table it may be concluded (granting the assumptions just stated) that both genetically male spores of each tetrad were polycladous and tufted. As to series d there is no such certainty, since of the 2 polycladous male spores both may have been tufted, or 1 tufted and 1 nontufted, the tuftedness or nontuftedness of 1 female spore not having been determined. Selecting, therefore, only those tetrads which can be classified with some confidence, the following results are obtained. (Abbreviations are those used in the tables.)

Of the two-type tetrads, that class composed of 2 ♀ np.nt. and 2 ♂ p.t. is represented by series a, b, and c in table 9, a total of 6 tetrads.

The class composed of 2 ♀ np.t. and 2 ♂ p.nt. is represented by series f, g, and h, a total of 10.

The class composed of 2 ♀ p.nt. and 2 ♂ np.t. is represented by the 1 tetrad of series n.

The class composed of 2 ♀ p.t. and 2 ♂ np.nt. is represented by series o and r—3 tetrads.

Thus all the predicted two-type classes are present. The numbers are

TABLE 9

Results of matings involving tuftedness and polyclady; distribution of offspring according to spore tetrads.

F_1 ♀	F_1 ♂	M.14	M.31	M.32	M.33	TOTALS	
(a) 2np.nt.	2p.	..	2	..	..	2	
(b) 2np.nt.	1p.	..	2	..	..	2	
(c) 2np.nt.	....	..	1	1	..	2	
(d) 1np.nt.	2p.	..	2	..	1	3	
(e) 1np.nt.	1p.	..	2	..	..	2	
(f) 2np.t.	2p.	..	2	2	..	4	
(g) 2np.t.	1p.	..	1	3	..	4	
(h) 2np.t.	....	..	..	2	..	2	
(i) 1np.t.	2p.	..	..	1	..	1	
(j) 1np.t.	1p.	..	..	3	..	3	
(k)	2p.	..	..	3	2	5	30
(l) 2p.	....	1	..	2	..	3	
(m) 1p.	1np.t.	..	..	1	..	1	
(n)	2np.t.	..	..	1	..	1	
(o) 2p.	2np.nt.	..	1	..	..	1	
(p) 2p.	1np.nt.	..	1	1	..	2	
(q) 1p.	1np.nt.	..	5*	..	..	5	
(r)	2np.nt.	..	2	..	..	2	15
(s) 1np.nt. / 1np.t.	....	..	2	..	..	2	
(t) 1np.nt. / 1p.	....	..	1	..	..	1	
(u) 1np.nt.	1np.nt.	..	1	..	..	1	
(v) 1np.t. / 1p.	1np.nt. / 1p.	..	..	1	..	1	
(w) 1np.t. / 1p.	1p.	..	..	1	..	1	
(x) 1np.t.	1np.nt. / 1p.	..	1	..	..	1	
(y) 2p.	1np.nt. / 1np.t.	..	..	1	..	1	
(z) 1p.	1np.nt. / 1p.	..	..	1	..	1	
(α) 1p.	1np.t. / 1p.	..	..	1	..	1	
(β) 1p.	1p.	..	..	1	..	1	11
Totals		1	26	26	3		56

* Including one cupulate male mutant.

small, but, as expected, those producing nonpolycladous females and polycladous males are more numerous than those producing polycladous females and nonpolycladous males.

Of the 8 possible four-type classes, at least 4 are represented, assuming the determinations of tufted and non-tufted clones to have been correctly made.

The 2 tetrads of series s belong to the class 1 ♀ np.nt., 1 ♀ np.t., 1 ♂ p.nt., 1 ♂ p.t.

The single tetrad of series y represents the class 1 ♀ p.nt., 1 ♀ p.t., 1 ♂ np. nt., 1 ♂ np.t.

The 1 tetrad of series u represents the class 1 ♀ np.nt., 1 ♀ p.t., 1 ♂ np.nt., 1 ♂ p.t.

The 1 tetrad of series v and the 1 of series x belong each to one or the other of the following classes: 1 ♀ np.t., 1 ♀ p.t., 1 ♂ np.nt., 1 ♂ p.nt.; or 1 ♀ np.t., 1 ♀ p.nt., 1 ♂ np.nt., 1 ♂ p.t.

In view of the limited number of definitely classifiable tetrads, it is striking that 8 of the 12 theoretically possible tetrad types are recognizably present, the missing ones being necessarily of infrequent occurrence. It can hardly be questioned that in larger families the remaining classes also would appear.

Repeated but unsuccessful attempts have been made to discover visible criteria for distinguishing as between polycladous clones of either sex those which are genetically tufted and those which are genetically nontufted.

As shown above, on the basis of the observed ratio between various possible classes of tetrads, the expected proportions of the different types of f_1 clones from matings of nonpolycladous × polycladous would be 9 ♀ nonpolycladous: 5 ♀ polycladous: 5 ♂ nonpolycladous: 9 ♂ polycladous. If the matings also involve tuftedness, introduced either by the female or by the male parent, tuftedness being inherited independently of either sex or polyclady, then each type of clone listed above would be replaced by nontufted and tufted types equal in number. But since polyclady masks tuftedness, the polycladous nontufted and polycladous tufted females would, phenotypically, constitute a single class, as would likewise polycladous nontufted and polycladous tufted males. The expected proportion, in a sufficiently large f_1 population, then, would be 9 ♀ nonpolycladous nontufted: 9 ♀ nonpolycladous tufted: 10 ♀ polycladous: 5 ♂ nonpolycladous nontufted: 5 ♂ nonpolycladous tufted: 18 ♂ polycladous. Table 10 shows the characters of all f_1 clones that were identifiable with reasonable confidence from families in which tuftedness and polyclady were involved.

The agreement between results and calculation is perhaps closer than might have been expected. Allowance must of course be made for the smaller numbers obtained of males than of females. The widest divergence from expectation is in the inequality between the first and second types of females. A reference to the proper categories of the partly identified clones listed at the foot of the table might bring the proportions of the respective types closer to expectation; it might, to be sure, conceivably work in the opposite direction.

TABLE 10

Results of matings involving polyclady and tuftedness; total fully identified offspring.

MATING	♀np.nt.	♀np.t.	♀p.	♂np.nt.	♂np.t.	♂p.	TOTALS
14	8	8	9	4	4	7	40
15	14	19	30	10	8	13	94
27	7	12	14	4	5	12	54
31	19	12	11	16	..	20	78
32	5	32	24	7	8	25	101
33	1	..	2	..	..	12	15
34	5	23	20	4	3	33	88
Totals	59	106	110	45	28	122	470
Expected Proportions	*9*	*9*	*10*	*5*	*5*	*18*	

♀nonpolycladous (nontufted or tufted?), 31.
♂nonpolycladous (nontufted or tufted?), 55.
Polycladous (undetermined as to sex, and as to nontuftedness or tuftedness), 8.

SUMMARY

1. Matings of nontufted×much-tufted result in the production of spore tetrads of which the majority yield, in about equal numbers of cases, respectively, 2♀nontufted and 2♂tufted, or 2♀tufted and 2♂nontufted. Of apparently "exceptional" tetrads, a considerable proportion would probably fall into one or the other of the 2 classes mentioned if all the genetically tufted clones could be recognized as such. A small number of tetrads, however, include each 3 or 4 genetically tufted spores; and probably an occasional one includes 3 or 4 genetically nontufted spores. There is evidence of the infrequent occurrence of four-type tetrads—each yielding 1♀nontufted, 1♀tufted, 1♂nontufted, and 1♂tufted.

2. Matings of little-tufted×little-tufted and of little-tufted×much-tufted produce, so far as available evidence shows, equal numbers of tetrads of the first 2 classes above mentioned.

3. Matings of much-tufted×much-tufted yield solely, or almost solely,

tufted offspring. Apparent exceptions were 1 female mutant, and 3 male clones which may really have been genetically tufted.

4. Among the offspring of each mating, individual clones differ greatly in the degree of expression of the tufted character.

5. In general, the average degree of tuftedness in an f_1 family varies in the same direction as the degree of tuftedness of the parents.

6. A hypothesis is developed for the explanation of the inheritance of tuftedness, involving multiple and complementary factors and certain assumptions regarding linkage.

7. This hypothesis may be simplified if it be supposed, as suggested by the results of certain matings involving 2 other characters, that factors derived from 1 parent may be inactivated, or their expression inhibited, by (possibly cytoplasmic) influences derived from the other parent.

8. Matings of non-polycladous × polycladous, whether or not other character-differences are involved, result in the production of tetrads of 3 classes, yielding respectively: 2 ♀ nonpolycladous, 2 ♂ polycladous; 2 ♀ polycladous, 2 ♂ nonpolycladous; and 1 ♀ nonpolycladous, 1 ♀ polycladous, 1 ♂ nonpolycladous, 1 ♂ polycladous. About twice as many of the first-named class of tetrads are produced as of the second; those of the third class (four-type tetrads) constitute about one sixth of the total number.

9. The proportions of tetrads in classes 1 and 2 indicate a partial linkage between polyclady (or its absence) and sex. Since the occurrence of crossing over between the large X- and the small Y-chromosome seems improbable, it is suggested that linkage in this case may imply a tendency for at least 2 of the chromosomes received from either parent to pass to the same pole in each meiotic division.

10. The occurrence of tetrads of the third class implies that some segregation of chromosomes or of chromosome parts occurs in the homoeotypic division. Reasons are given for concluding that segregation does not take place exclusively in that division; the observed facts are explainable either by the segregation of 1 chromosome pair in the heterotypic, and of another, regularly or occasionally, in the homoeotypic division, or by a crossing over in the four-strand stage.

11. From the relative numerical occurrence respectively of tetrads of the 3 classes, it is calculated that the 4 possible clonal types should appear in a sufficiently large f_1 population in the following proportions: 9 ♀ nonpolycladous: 5 ♀ polycladous: 5 ♂ nonpolycladous: 9 ♂ polycladous. A tabulation of all f_1 progeny of matings involving polyclady shows a close agreement with the calculated ratio on the part of female clones, but a considerable divergence on the part of males. It is pointed out that, for vari-

ous reasons noted, the classification of female is more reliable than that of male clones.

12. In families to which tuftedness and polyclady were contributed, each by 1 parent, if the 2 characters are separately inherited, apart from certain combinations necessarily infrequent, 4 classes of two-type tetrads and 8 classes of four-type tetrads would be expected. A difficulty is interposed by the masking of the tufted character by polyclady; but the available facts indicate that all 4 expected classes of two-type tetrads and at least 4 of the 8 expected classes of four-type tetrads are produced.

13. In the work reported in the present paper, 1273 f_1 clones have been determined as to both sex and vegetative character. Among these were 5 apparent mutants: 2 appendiculate females, 1 polycladous female, 1 dwarf male, and 1 cupulate male. The 2 male mutants represent forms that have never otherwise been observed.

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